Carrageenan-Volume 2 #14 8/11/72

CARRAGEENAN VOL II #14

M O N O G R A P H

O N

C A R R A G E E N A N

V o l. II

TR-72-1552-03

Submitted Under:
Contract No. FDA 72-104

August 11, 1972

INFORMATICS INC.
6000 Executive Boulevard
Rockville, Maryland 20852

Food and Drug Research Laboratories
INCORPORATED

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FINAL REPORT

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date May 1, 1972

Laboratory No. 0728 f
Contract No. FDA 71-260

Sample: Fine tan powdered material

Marking: FDA 71-5 (Calcium carragheenate)

Examination Requested: Teratologic evaluation of FDA 71-5 in mice.

Procedure: See Appendix I

Results: See Tables 1 through 4 and Appendix II

Conclusion: Subject to reexamination in the light of later findings, the following is concluded:

"The administration of the test material in graded dosage levels up to 900 mg/kg (body weight) to pregnant mice for 10 consecutive days caused an apparent increase in the number of resorptions and/or fetal deaths in utero. There was a corresponding decrease in the number of live pups and a reduction in pup weight at delivery, both of which appear to have been dose-dependent. A concurrent retardation in skeletal maturation may be inferred from the increased incidence of missing sternebrae and incomplete skull closure. All other soft and skeletal tissue abnormalities were probably within the accepted limits of variability for the species."

It was concluded that the test material was fetotoxic in the pregnant mouse without exhibiting frank teratogenicity.

(See following page for comment)

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Kenneth Morgareidge

Kenneth Morgareidge, Ph.D.
Vice President

Comment: Attention is called to the fact that this is the fourth of a series of reports which will be issued in accordance with the terms of the contract cited above. Eventually, a total of at least 36 compounds will have been tested in 18 pairs; each pair being run concurrently against one sham-treated control and one positive control group. Because of the inherent variability of biological data of the type dealt with here, the accumulation and pooling of sequential sets of control values will greatly enhance the statistical value of the findings and the ultimate reliability of the test results.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups: 31 & 32; 37 through 40

Material: FDA 71-5

Date March 31, 1972

Laboratory No. 0728 f

Table 1

Fate Summary
(Mice)

Group	Material	Dose mg/kg	Total		At Term	
			Mated	Pregnant	Surviving (Total)	Number Pregnant
31	Sham	0	27	27	26	26
32	Aspirin*	150	26	24	22	21
37	FDA 71-5	10	25	24	25	24
38	FDA 71-5	45	28	27	26	25
39	FDA 71-5	470	28	23	28	23
40	FDA 71-5	900	40	30	30	25

* Positive Control

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group: 31 & 32; 37 through 40

Material: FDA 71-5

Date March 31, 1972

Laboratory No. 0728 f

Table 2
Reproduction Data
(Mice)

Group:	31	32	37	38	39	40
Dose (mg/kg):	Sham	Aspirin**	10	45	470	900
Pregnancies						
Total No.	27	24	24	27	23	30
Died or Aborted (before Day 17)	1	4	0	2	0	10
To term (on Day 17)	26	21	24	25	23	25
Live litters						
Total No. *	26	20	24	25	22	23
Implant sites						
Total No.	306	241	282	299	281	279
Average/dam *	11.8	11.5	11.8	12.0	12.2	11.2
Resorptions						
Total No. *	10	21	11	10	20	35
Dams with 1 or more sites resorbed	4	7	8	6	8	12
Dams with all sites resorbed	0	1	0	0	1	2
Per cent partial resorptions	15.4	33.3	33.3	24.0	34.8	48.0
Per cent complete resorptions	-	4.76	-	-	4.35	8.0
Live fetuses						
Total No.	296	218	268	285	258	243
Average/dam *	11.4	10.4	11.2	11.4	11.2	9.72
Dead fetuses						
Total No. *	0	2	3	4	3	1
Dams with 1 or more dead	-	2	3	3	2	1
Dams with all dead	-	0	0	0	0	-
Per cent partial dead	-	9.52	12.5	12.0	8.70	4.0
Per cent all dead	-	-	-	-	-	-
Average fetus weight, g	0.96	0.92	1.03	0.92	0.95	0.80

* Includes only those dams examined at term.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 31 & 32; 37 through 40 Laboratory No. 0728 f

Table 3

Material FDA 71-5 Date March 31, 1972

Summary of Skeletal Findings*

(Mice)

Findings	Group No.	31	32	37	38	39	40
	Dose (mg/kg)	Sham	Aspirin**	10	45	470	900
Live Fetuses Examined		193/25	151/20	185/24	197/25	179/22	163/2
Sternebrae							
Incomplete oss.		23/13	44/16	35/13	62/21	31/11	46/1
Scrambled				1/1	1/1		
Bipartite		5/3	11/7	13/6	13/9	8/6	31/1
Fused							
Extra				2/1		1/1	
Missing		4/3	2/2	4/3	18/5	13/4	34/9
Ribs							
Incomplete oss.							
Fused/split				1/1			
Wavy							1/1
Less than 12							
More than 13		16/9	7/5	13/6	16/8	14/7	2/2
Other							
Vertebrae							
Incomplete oss.				1/1	6/2		5/3
Scrambled				5/2			
Fused							
Extra ctrs. oss.		1/1					
Scoliosis				1/1			
Tail defects							
Other							
Skull							
Incomplete closure			2/1	6/4		12/4	8/3
Missing							
Craniostosis							
Other; exencephaly		1/1				1/1	
Extremities							
Incomplete oss.					5/2	3/1	
Missing							
Extra							
Miscellaneous							
Hyoid; reduced		8/5	14/8	9/7	36/19	15/10	15/9
Hyoid; missing		8/7	19/9	10/6	18/11	24/11	38/1

* Numerator=Number of fetuses affected; Denominator=Number of litters affecte

** Positive control: 150 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 31 & 32; 37 through 40 Date March 31, 1972

Material FDA 71-5 Laboratory No. 0728 f

Table 3-a

Summary of Soft Tissue Abnormalities

(Mice)

Group	Material	Dose level mg/kg	Dam	Number of Pups	Description
32	Aspirin	150	A-8104	1	Fetal monster
				1	Anopia
				1	Mouth and nasal absent

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 31 & 32; 37 through 40

Species Mice

Table 4

Average Body Weights

Date March 31, 1972

Laboratory No. 0728 f

Group	Material	Dose Level	Day 0	6	11	15	17	**
		mg/kg	g					
31	Sham	0	26.6	29.8	34.0	40.2	48.1	(26)
32	Aspirin	150	26.8	30.0	33.0	37.9	43.6	(21)
37	FDA 71-5	10	27.6	30.0	34.8	44.5	49.5	(24)
38	FDA 71-5	45	27.6	29.3	33.8	41.5	48.6	(25)
39	FDA 71-5	470	28.8	31.7	33.4	40.3	47.0	(23)
40	FDA 71-5	900	27.8	29.8	30.2	34.5	39.8	(25)

* Of pregnant dams
** Number of surviving dams in parentheses (c.f. Table 1)

Appendix I

Teratology Study in Mice

Virgin adult female albino CD-1 outbred mice were individually housed in disposable plastic cages in temperature and humidity-controlled quarters with free access to food and fresh tap water. They were mated with young adult males, and observation of the vaginal sperm plug was considered Day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, the females were dosed with the indicated dosages by oral intubation; the controls were sham treated.

Body weights were recorded on Days 0, 6, 11, 15, and 17 of gestation. All animals were observed daily for appearance and behavior with particular attention to food consumption and weight, in order to rule out any abnormalities which may have occurred as a result of anorexic effects in the pregnant female animal.

On Day 17 all dams were subjected to Caesarean section under surgical anesthesia, and the numbers of implantation sites, resorption sites, and live and dead fetuses were recorded. The body weights of the live pups were also recorded. The urogenital tract of each dam was examined in detail for anatomical normality.

All fetuses were examined grossly for the presence of external congenital abnormalities. One-third of the fetuses of each litter underwent detailed visceral examinations employing 10X magnification. The remaining two-thirds were cleared in potassium hydroxide (KOH), stained with alizarin red S dye and examined for skeletal defects.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 31 | Appendix II | Date March 31, 1972

Material Sham | Reproduction Data in Mice (Individual) | Laboratory No. 0728

Dose 0

Dam No.	Fate *	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
S 8091	P	11	9		2	0.92	
S 8092	P	14		14		----	Died Day 15
S 8093	P	14	14			0.97	
S 8094	P	13	10		3	0.94	
S 8095	P	13	13			0.85	
S 8096	P	14	14			0.93	
S 8097	P	16	16			0.95	
S 8098	P	11	11			0.96	
S 8099	P	13	13			0.97	
S 8100	P	13	13			0.90	
S 8101	P	8	8			0.99	
S 8102	P	12	12			1.11	
S 8103	P	8	8			0.97	
S 8104	P	11	11			0.91	
S 8105	P	10	10			0.95	
S 8106	P	13	13			0.89	
S 8107	P	13	13			0.88	
S 8108	P	11	11			0.92	
S 8109	P	13	12		1	0.99	
S 8110	P	15	15			1.04	
S 8111	P	15	15			1.06	
S 8112	P	9	9			1.02	
S 8113	P	12	12			0.98	
S 8114	P	3	3			1.08	
S 8115	P	13	13			0.87	
S 8116	P	12	8		4	1.04	
S 8117	P	10	10			0.87	

* P= Pregnant; NP= Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 32 | Appendix II | Date March 31, 1972

Material Aspirin | Reproduction Data in Mice (Individual) | Laboratory No. 0728

Dose 150 mg/kg

Dam No.	Fate *	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
A 8091	P	14		13	1	----	Died Day 16
A 8092	P	15		14	1	----	Died Day 16
A 8093	P	12	12			0.69	
A 8094	P	11	11			0.96	
A 8095	P	15			15	----	
A 8096	P	11	10		1	0.95	
A 8097	P	13	12		1	0.91	
A 8098	P	10	10			0.84	
A 8099	P	12	12			0.85	
A 8100	P	14	14			0.85	
A 8101	P	10	9		1	1.22	
A 8102	P	10	10			1.02	
A 8103	P	15	14	1		0.89	
A 8104	P	10	9	1		0.90	
A 8105	NP	0				----	Died Day 13
A 8106	--	--				----	Number not assigned.
A 8107	P	11	10		1	0.88	
A 8108	P	12	12			0.84	
A 8109	P	12	12			1.01	
S 8110	P	12		12		----	Died Day 14
S 8111	P	11	10		1	0.96	
S 8112	P	8	8			0.91	
S 8113	P	10	10			0.92	
S 8114	P	12	12			1.00	
S 8115	P	9	8		1	0.93	
S 8116	NP	0				----	
S 8117	P	13	13			0.93	

* P= Pregnant; NP= Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 37 | Appendix II | Date March 31, 1972

Material FDA 71-5 | Reproduction Data in Mice (Individual) | Laboratory No. 0728 f

Dose 10 mg/kg

Dam No.	Fate *	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
F 8001	P	11	11			0.89	
F 8002	P	13	13			0.97	
F 8003	P	14	14			1.01	
F 8004	P	11	11			0.93	
F 8005	P	16	16			0.81	
F 8006	P	12	11		1	0.86	
F 8007	P	13	13			0.99	
F 8008	P	10	9		1	1.30	
F 8009	NP	0				----	
F 8010	P	11	10		1	1.01	
F 8011	P	15	14	1		1.04	
F 8012	P	9	7	1	1	1.41	
F 8013	P	11	10		1	1.12	
F 8014	P	7	4		3	0.88	
F 8015	P	12	12			0.86	
F 8016	P	11	11			1.12	
F 8017	P	10	10			1.01	
F 8018	P	11	11			1.27	
F 8019	P	14	14			0.88	
F 8020	P	13	13			1.20	
F 8021	P	10	10			1.28	
F 8022	P	13	12		1	1.28	
F 8023	P	12	12			0.80	
F 8024	P	13	12	1		0.86	
F 8025	P	10	8		2	0.84	

* P= Pregnant; NP= Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 38 | Appendix II | Date March 31, 1972

Material FDA 71-5 | Reproduction Data in Mice (Individual) | Laboratory No. 0728 f

Dose 45 mg/kg

Dam No.	Fate *	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
F 8031	P	13	11	2		0.95	
F 8032	P	14	14			0.99	
F 8033	P	11	10	1		0.97	
F 8034	P	14		14		----	Died Day 17
F 8035	P	10	10			1.02	
F 8036	P	9			9	----	Died Day 15
F 8037	-	-				----	Number not assigned.
F 8038	P	13	13			0.93	
F 8039	P	9	9			0.80	
F 8040	P	11	10	1		0.76	
F 8041	P	9	9			0.98	
F 8042	P	11	11			0.97	
F 8043	P	9	9			0.98	
F 8044	P	11	10		1	0.94	
F 8045	NP	0				----	
F 8046	P	11	10		1	1.05	
F 8047	P	12	12			1.06	
F 8048	P	10	8		2	1.06	
F 8049	P	12	11		1	0.93	
F 8050	P	18	18			0.85	
F 8051	P	15	15			0.94	
F 8052	P	11	11			0.61	
F 8053	P	12	12			0.98	
F 8054	P	11	11			0.99	
F 8055	P	12	12			0.86	
F 8056	P	11	11			0.93	
F 8057	P	15	15			0.87	
F 8058	P	13	9		4	0.75	
F 8059	P	15	14		1	0.77	

* P= Pregnant; NP= Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 39 | Appendix II | Date March 31, 1972

Material FDA 71-5 | Reproduction Data in Mice (Individual) | Laboratory No. 0728 f

Dose 470 mg/kg

Dam No.	Fate *	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
F 8061	P	13			13	----	
F 8062	P	15	15			0.95	
F 8063	P	14	14			0.88	
F 8064	P	10	10			0.97	
F 8065	P	13	13			0.93	
F 8066	P	13	13			0.87	
F 8067	P	10	9		1	0.85	
F 8068	NP	--	--			----	
F 8069	P	13	13			0.74	
F 8070	P	12	12			0.94	
F 8071	P	7	6		1	0.91	
F 8072	NP	0	--			----	
F 8073	NP	0	-			----	
F 8074	P	11	11			1.04	
F 8075	P	12	12			0.92	
F 8076	NP	--	--			----	
F 8077	P	12	11		1	1.03	
F 8078	P	12	11		1	0.90	
F 8079	P	17	17			0.85	
F 8080	P	12	10	2		1.09	
F 8081	P	12	12			0.93	
F 8082	P	12	11		1	1.18	
F 8083	NP	0				----	
F 8084	P	13	13			1.20	
F 8085	P	12	12			1.24	
F 8086	P	13	11	1	1	0.55	
F 8087	P	13	12		1	0.78	
F 8088	P	10	10			1.08	

* P= Pregnant; NP= Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 40 | Appendix II | Date March 31, 1972

Material FDA 71-5 | Reproduction Data in Mice (Individual) | Laboratory No. 0728 f

Dose 900 mg/kg

Dam No.	Fate *	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
F 8091	P	14	14			0.64	
F 8092	P	12	12			0.85	
F 8093	P	11	11			0.68	
F 8094	P	14	14			0.85	
F 8095	P	16	1		15	----	
F 8096	NP	--				----	
F 8097	P	8			8	----	
F 8098	P	11	10		1	0.80	
F 8099	NP	--				----	Died Day 13
F 8100	P	12	11		1	0.62	
F 8101	P	10	9		1	1.05	
F 8102	P	10		10		----	Died Day 12
F 8103	NP	--				----	
F 8104	P	9	8		1	0.96	
F 8105	NP	--				----	Died Day 14
F 8106	NP	--				----	
F 8107	P	10	10			0.97	
F 8108	P	6	6			0.76	
F 8109	NP	--				----	Died Day 12
F 8110	P	12		12		----	Died Day 15
F 8111	P	9	9			0.68	
F 8112	P	10		8	2	----	Died Day 15
F 8113	P	13	12		1	0.85	
F 8114	P	8	8			0.60	
F 8115	P	13	13			0.72	
F 8116	P	7			7	----	Died Day 11
F 8117	P	9	9			----	Aborted Day 16
F 8118	P	11	11			0.79	
F 8119	P	11	10		1	0.61	

* P= Pregnant; NP= Not Pregnant

(continued on following page)

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 40 (concluded) | Appendix II | Date March 31, 1972

Material FDA 71-5 | Reproduction Data in Mice (Individual) | Laboratory No. 0728 f

Dose 900 mg/kg

Dam No.	Fate *	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
F 8120	P	2			2	----	
F 8121	P	9	8	1		0.94	
F 8122	NP	--				----	
F 8123	P	12	11		1	0.92	
F 8124	P	15	13		2	0.88	
F 8125	P	14	14			0.73	
F 8126	NP	--				----	Died Day 12.
F 8127	NP	--				----	
F 8128	P	13	12		1	0.94	
F 8129	NP	--				----	Died Day 12
F 8130	P	16	16			0.80	

* P= Pregnant; NP= Not Pregnant

Food and Drug Research Laboratories
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FINAL REPORT

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date May 1, 1972

Laboratory No. 0729 f
Contract No. FDA 71-260

Sample: Fine tan powdered material

Marking: FDA 71-5 (Calcium carragheenate)

Examination Requested: Teratologic evaluation of FDA 71- 5 in rats

Procedure: See Appendix I

Results: See Tables 1 through 4 and Appendix II

Conclusion: Subject to reexamination in the light of later findings, the following is concluded:

"The administration of the test material in graded dosage levels up to 600 mg/kg (body weight) to pregnant rats for 10 consecutive days caused an apparent increase in the number of resorption sites observed with or without a corresponding decrease in the number of live pups delivered. At the highest dose level, there may have been a decrease in the birth weight of the pups. A concurrent retardation in skeletal maturation was indicated by a dose-dependent increase in missing sternebrae. There were no other findings in either soft or skeletal tissues which appeared to be treatment-related.

It was concluded that the test material depressed fetal development in the pregnant rat and caused an increase in early fetal deaths (resorptions). There was no evidence of frank teratogenicity.

(See following page for comment)

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Kenneth Morgareidge

Kenneth Morgareidge, Ph.D.
Vice President

Comment: Attention is called to the fact that this is the fourth of a series of reports which will be issued in accordance with the terms of the contract cited above. Eventually, a total of at least 36 compounds will have been tested in 18 pairs; each pair being run concurrently against one sham-treated control and one positive control group. Because of the inherent variability of biological data of the type dealt with here, the accumulation and pooling of sequential sets of control values will greatly enhance the statistical value of the findings and the ultimate reliability of the test results.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups: 31 & 32; 37 through 40

Material: FDA 71-5

Date March 31, 1972

Laboratory No. 0729 f

Table 1

Fate Summary
(Rats)

Group	Material	Dose mg/kg	Total		At Term	
			Mated	Pregnant	Surviving (Total)	Number Pregnant
31	Sham	0	25	23	25	23
32	Aspirin*	250	29	27	25	24
37	FDA 71-5	40	24	23	23	22
38	FDA 71-5	100	24	22	24	22
39	FDA 71-5	240	25	22	22	20
40	FDA 71-5	600	28	21	24	19

* Positive Control

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group: 31 & 32; 37 through 40

Date March 31, 1972

Material: FDA 71-5

Table 2
Reproduction Data
(Rats)

Laboratory No. 0729 f

Group:	31	32	37	38	39	40
Dose (mg/kg):	Sham	Aspirin**	40	100	240	600
Pregnancies						
Total No.	23	27	23	22	22	21
Died or Aborted (before Day 20)	0	4	1	0	3	4
To term (on Day 20)	23	24	22	22	20	19
Live litters						
Total No. *	23	18	22	22	20	19
Implant sites						
Total No.	252	267	233	246	233	184
Average/dam *	11.0	11.1	10.6	11.2	11.7	9.68
Resorptions						
Total No. *	7	110	9	4	6	21
Dams with 1 or more sites resorbed	5	15	6	4	6	8
Dams with all sites resorbed	0	5	0	0	0	0
Per cent partial resorptions	21.7	62.5	27.3	16.0	30.0	42.1
Per cent complete resorptions	-	20.8	-	-	-	-
Live fetuses						
Total No.	245	156	224	241	226	162
Average/dam *	10.7	6.50	10.2	11.0	11.3	8.53
Dead fetuses						
Total No. *	0	1	0	1	1	1
Dams with 1 or more dead	-	1	-	1	1	1
Dams with all dead	-	0	-	0	0	0
Per cent partial dead	-	4.17	-	4.55	5.00	5.26
Per cent all dead	-	0	-	-	-	-
Average fetus weight, g	3.75	2.47	3.99	3.91	3.70	3.43

* Includes only those dams examined at term

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 31 & 32; 37 through 40 — Laboratory No. 0729 f

Table 3

Material FDA 71-5 — Date March 31, 1972

Summary of Skeletal Findings*

(Rats)

Findings	Group No.	31	32	37	38	39	40
	Dose (mg/kg)	Sham	Aspirin**	40	100	240	600
Live Fetuses Examined		171/23	103/17	155/22	168/22	157/22	112/19
Sternebrae							
Incomplete oss.		93/23	41/15	73/20	149/22	104/19	81/20
Scrambled		18/11	16/6	16/11	62/21	41/19	31/14
Bipartite		16/10	27/10	7/7		21/10	4/4
Fused							
Extra						3/1	
Missing		23/10	91/17	30/10	8/6	28/8	45/12
Other							
Ribs							
Incomplete oss.			2/2				
Fused/split			3/3			1/1	
Wavy		1/1	18/8		4/3		9/5
Less than 12						1/1	
More than 13		2/2	19/7	1/1			
Other							
Vertebrae							
Incomplete oss.			9/5			2/1	
Scrambled			15/7			6/3	
Fused						1/1	
Extra ctrs. oss.							
Scoliosis			1/1			1/1	3/3
Tail defects							
Other							
Skull							
Incomplete closure		19/12	10/3	43/17	5/5	19/7	10/6
Missing						2/1	
Craniostosis		2/1					
Other							
Extremities							
Incomplete oss.			1/1				
Missing			1/1				
Extra							
Miscellaneous							
Hyoid; reduced		12/7	28/6	14/8	23/10	18/13	10/7
Hyoid; missing		11/6	51/13	17/11	7/5	13/10	23/1

* Numerator=Number of fetuses affected; Denominator=Number of litters affect
** Positive control: 250 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 31 & 32; 37 through 40 Date March 31, 1972

Material FDA 71-5 Laboratory No. 0729 f

Table 3-a

Summary of Soft Tissue Abnormalities

(Rats)

Group	Material	Dose level mg/kg	Dam	Number of Pups	Description
32	Aspirin	250	A-9091	1	Meningoencephalocele
32	Aspirin	250	A-9104	6	Anopia
				5	Club feet
				6	Hydrocephalus
				6	Umbilical hernia
				5	Cleft palate
				6	Meningoencephalocele

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 31 & 32; 37 through 40 | Date March 31, 1972

Species Rats | Laboratory No. 0729 f

Table 4

Average Body Weights *

Group	Material	Dose Level	Day 0	6	11	15	20	**
		mg/kg	g					
31	Sham	0	216	236	250	270	322	(23)
32	Aspirin	250	230	247	254	269	308	(24)
37	FDA 71-5	40	211	228	243	263	322	(22)
38	FDA 71-5	100	221	238	252	271	337	(22)
39	FDA 71-5	240	215	238	251	265	330	(20)
40	FDA 71-5	600	212	234	236	243	301	(19)

* Of pregnant dams
** Number of surviving dams in parentheses (c.f. Table 1)

Appendix I

Teratology Study in Rats

Virgin adult female albino rats (Wistar derived stock) were individually housed in mesh bottom cages in temperature and humidity-controlled quarters with free access to food and fresh tap water. They were mated with young adult males, and observation of the vaginal sperm plug was considered Day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, the females were dosed with the indicated dosages by oral intubation; the controls were sham treated.

Body weights were recorded on Days 0, 6, 11, 15, and 20 of gestation. All animals were observed daily for appearance and behavior with particular attention to food consumption and weight, in order to rule out any abnormalities which may have occurred as a result of anorexic effects in the pregnant female animal.

On Day 20 all dams were subjected to Caesarean section under surgical anesthesia, and the numbers of implantation sites, resorption sites, and live and dead fetuses were recorded. The body weights of the live pups were also recorded. The urogenital tract of each dam was examined in detail for anatomical normality.

All fetuses were examined grossly for the presence of external congenital abnormalities. One-third of the fetuses of each litter underwent detailed visceral examinations employing 10X magnification. The remaining two-thirds were cleared in potassium hydroxide (KOH), stained with alizarin red S dye and examined for skeletal defects.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 31 | Appendix II | Date March 31, 1972

Material Sham | Reproduction Data in Rats (Individual) | Laboratory No. 0729

Dose 0

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
S 9091	P	11	11			4.09	
S 9092	P	11	11			3.84	
S 9093	P	9	9			4.13	
S 9094	P	12	12			3.70	
S 9095	P	12	12			3.36	
S 9096	P	11	11			3.64	
S 9097	P	6	4		2	4.17	
S 9098	P	9	9			3.71	
S 9099	NP	0				----	
S 9100	P	14	14			3.44	
S 9101	P	14	13		1	3.88	
S 9102	P	7	7			4.13	
S 9103	P	11	11			3.93	
S 9104	P	10	8		2	3.32	
S 9105	P	8	8			3.43	
S 9106	P	12	12			4.02	
S 9107	P	10	10			4.12	
S 9108	NP	0				----	
S 9109	P	12	12			3.95	
S 9110	P	10	9		1	3.98	
S 9111	P	8	8			3.92	
S 9112	P	12	12			3.90	
S 9113	P	14	14			3.83	
S 9114	P	11	11			3.44	
S 9115	P	18	17		1	2.32	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 32 — Appendix II — Date March 31, 1972

Material Aspirin — Reproduction Data in Rats (Individual) — Laboratory No. 0729

Dose 250 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
A 9091	P	13	8		5	2.15	
A 9092	P	12			12	----	
A 9093	P	11		11		----	Died Day 7.
A 9094	P	13	1		12	1.79	
A 9095	P	11	2		9	1.76	
A 9096	P	14	14			3.12	
A 9097	P	14	14			3.07	
A 9098	P	12			12	----	
A 9099	NP	0				----	
A 9100	P	13	13			2.58	
A 9101	P	13			13	----	
A 9102	P	12	10		2	2.20	
A 9103	P	7	1		6	1.70	
A 9104	P	10	8		2	1.75	
A 9105	P	11		1	10	----	
A 9106	P	15	10		5	2.36	
A 9107	P	9	9			2.60	
A 9108	P	10	10			2.49	
A 9109	P	11	11			2.55	
A 9110	P	6			6	----	
A 9111	P	11	11			3.86	
A 9112	NP	0				----	Died Day 8.
A 9113	P	11	8		3	2.83	
A 9114	P	12		12		----	
A 9115	P	11	11			2.66	
A 9116	P	9	9			2.49	
A 9117	P	14			14	----	
A 9118	P	8	6		2	2.51	
A 9120	P	11			11	----	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 37 | Appendix II | Date March 31, 1972

Material FDA 71-5 | Reproduction Data in Rats (Individual) | Laboratory No. 0729 f

Dose 40 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
F 9001	P	11	11			3.87	
F 9002	P	10	10			3.74	
F 9003	P	10	10			3.88	
F 9004	P	13	12		1	4.28	
F 9005	P	10	10			3.41	
F 9006	NP	0				----	
F 9007	P	5	5			3.76	
F 9008	P	14	12		2	3.43	
F 9009	P	8	8			3.84	
F 9010	P	11	11			3.79	
F 9011	P	13	13			4.05	
F 9012	P	14	12		2	3.99	
F 9013	P	10	10			4.02	
F 9014	P	12	11		1	3.91	
F 9015	P	6	6			4.34	
F 9016	P	11	11			4.35	
F 9017	P	9	9			5.66	
F 9018	P	10		10		----	Died Day 20.
F 9019	P	11	11			3.92	
F 9020	P	11	11			3.84	
F 9021	P	9	9			4.17	
F 9022	P	9	9			4.13	
F 9023							Number not assigned
F 9024							Number not assigned
F 9025	P	13	12		1	4.08	
F 9026	P	13	11		2	3.25	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 38 | Appendix II | Date March 31, 1972

Material FDA 71-5 | Reproduction Data in Rats (Individual) | Laboratory No. 0729 f

Dose 100 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
F 9031	P	9	9			3.70	
F 9032	P	11	11			3.79	
F 9033	P	13	12		1	4.16	
F 9034	P	14	14			3.58	
F 9035	P	13	13			4.17	
F 9036	P	8	8			3.77	
F 9037	P	11	10	1		3.89	
F 9038	NP	0				----	
F 9039	P	13	13			3.87	
F 9040	P	10	10			4.09	
F 9041	P	10	10			3.70	
F 9042	P	13	13			4.01	
F 9043	P	11	10		1	3.94	
F 9044	P	9	8		1	3.73	
F 9045	P	12	12			4.02	
F 9046	P	10	10			3.94	
F 9047	P	10	9		1	4.07	
F 9048	P	13	13			4.03	
F 9049	P	8	8			3.99	
F 9050	P	9	9			4.21	
F 9051	NP	0				----	
F 9052	P	15	15			3.71	
F 9053	P	14	14			3.85	
F 9054	P	10	10			3.81	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Appendix II

Reproduction Data in Rats (Individual)

Group 39

Material FDA 71-5

Dose 240 mg/kg

Date March 31, 1972

Laboratory No. 0729 f

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
F 9061	P	11	11			4.35	
F 9062	P	12	12			4.28	
F 9063	P	14	13		1	3.78	
F 9064	P	13	13			3.93	
F 9065	P	9	8		1	2.71	
F 9066	P	13	13			3.55	
F 9067	P	11	10		1	3.37	
F 9068	P	11	10	1		3.15	
F 9069	P	12	12			3.18	
F 9070	P	10	10			3.75	
F 9071	NP	0				----	
F 9072	P	14	14			3.73	
F 9073	P	10	9		1	4.23	
F 9074	P	15	15			3.49	
F 9075	NP	0				----	
F 9076	P	12	12			3.89	
F 9077	P	9			9	----	Died Day 20.
F 9078	P	12	12			3.74	
F 9079	P	10	10			3.80	
F 9080	P	11	10		1	4.13	
F 9081	P	12		12		----	Died Day 20.
F 9082	P	11	11			3.99	
F 9083	P	13	13			3.85	
F 9084	P	9	8		1	3.03	
F 9085	NP	0				----	Died Day 15.

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 40 | Appendix II | Date March 31, 1972

Material FDA 71-5 | Reproduction Data in Rats (Individual) | Laboratory No. 0729 f

Dose 600 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
F 9091	P	9	9			4.29	
F 9092	P	11	11			3.66	
F 9093	P	13	13			3.72	
F 9094	NP	0				----	Died Day 8.
F 9095	NP	0				----	
F 9096	P	2	2			3.78	
F 9097	P	9	8		1	3.71	
F 9098	NP	0				----	
F 9099	P	10	9		1	4.07	
F 9100	NP	0				----	
F 9101	P	7		7		----	Died Day 19.
F 9102	NP	0				----	
F 9103	P	12	8		4	2.42	
F 9104	P	4	4			3.96	
F 9105	P	9	9			2.54	
F 9106	P	7	7			3.07	
F 9107	P	6	6			3.84	
F 9108	P	10	10			4.18	
F 9109	NP	0				----	
F 9110	P	11	10		1	3.63	
F 9111	P	10	3		7	1.99	
F 9112	P	9	8		1	3.77	
F 9113	P	11		11		----	Died Day 14.
F 9114	P	14	14			3.75	
F 9115	P	13	13			3.19	
F 9116	P	11	5	1	5	2.41	
F 9117	NP	0				----	Died Day 7.
F 9118	P	14	13		1	3.14	

* P = Pregnant; NP = Not Pregnant

Food and Drug Research Laboratories
INCORPORATED

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FINAL REPORT

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date May 1, 1972

Laboratory No. 0730 f
Contract No. FDA 71-260

Sample: Fine tan powdered material

Marking: FDA 71-5 (Calcium carragheenate)

Examination Requested: Teratologic evaluation of FDA 71- 5 in hamsters

Procedure: See Appendix I

Results: See Tables 1 through 4 and Appendix II

Conclusion: Subject to reexamination in the light of later findings, the following is concluded:

"The administration of the test material in graded dosage levels to pregnant hamsters for 5 consecutive days had no clearly significant effect on nidation or on maternal or fetal survival. There was some evidence of delayed skeletal maturation (missing or incomplete centers of ossification) which was dose dependent. The number of malformations (terata?) seen were within the range of normal variation for the species, except for the occurrence of extra ribs. The conclusion is less clear than in the case of the sodium salt of carragheenin in the same species.

(See following page for comment)

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Kenneth Morgareidge

Kenneth Morgareidge, Ph.D.
Vice President

Comment: Attention is called to the fact that this is the fourth of a series of reports which will be issued in accordance with the terms of the contract cited above. Eventually, a total of at least 36 compounds will have been tested in 18 pairs; each pair being run concurrently against one sham-treated control and one positive control group. Because of the inherent variability of biological data of the type dealt with here, the accumulation and pooling of sequential sets of control values will greatly enhance the statistical value of the findings and the ultimate reliability of the test results.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups: 31 & 32; 37 through 40

Material: FDA 71-5

Date March 31, 1972

Laboratory No. 0730 f

Table 1

Fate Summary
(Hamsters)

Group	Material	Dose mg/kg	Total		At Term	
			Mated	Pregnant	Surviving (Total)	Number Pregnant
31	Sham	0	30	30	29	29
32	Aspirin*	250	30	27	29	26
37	FDA 71-5	40	26	23	26	23
38	FDA 71-5	100	26	25	26	25
39	FDA 71-5	240	27	24	26	23
40	FDA 71-5	600	30	30	29	29

* Positive Control

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group: 31 & 32; 37 through 40 Date March 31, 1972

Material: FDA 71-5 Laboratory No. 0730 f

Table 2
Reproduction Data
(Hamsters)

Group:	31	32	37	38	39	40
Dose (mg/kg):	Sham	Aspirin**	40	100	240	600
Pregnancies						
Total No.	30	27	23	25	24	29
Died or Aborted (before Day 14)	1	1	0	0	1	1
To term (on Day 14)	29	26	23	25	23	29
Live litters						
Total No. *	29	24	23	25	21	28
Implant sites						
Total No.	340	327	278	324	290	361
Average/dam *	11.7	12.6	12.1	13.0	12.6	12.4
Resorptions						
Total No. *	15	31	10	12	16	20
Dams with 1 or more sites resorbed	8	12	7	8	6	12
Dams with all sites resorbed	0	2	0	0	1	1
Per cent partial resorptions	27.6	46.2	30.4	32.0	26.1	41.4
Per cent complete resorptions	-	7.69	-	-	4.35	3.45
Live fetuses						
Total No.	322	296	268	312	260	341
Average/dam *	11.1	11.4	11.7	12.5	11.3	11.8
Dead fetuses						
Total No. *	3	-	-	-	14	0
Dams with 1 or more dead	3	-	-	-	2	-
Dams with all dead	0	-	-	-	1	-
Per cent partial dead	10.3	-	-	-	8.70	-
Per cent all dead	-	-	-	-	4.35	-
Average fetus weight, g	1.86	1.78	1.78	1.78	1.82	1.77

* Includes only those dams examined at term.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 31 & 32; 37 through 40 — Table 3 — Laboratory No. 0730 f

Material FDA 71-5 — Date March 31, 1972

Summary of Skeletal Findings *
(Hamsters)

Findings	Group No.	31	32	37	38	39	40
	Dose (mg/kg)	Sham	Aspirin**	40	100	240	600
Live Fetuses Examined (at term)		220/29	206/24	185/23	210/24[a]	181/21	235/28
Sternebrae							
Incomplete oss.		75/27	92/24	81/21	60/21	41/14	44/18
Scrambled		1/1					
Bipartite		30/19	26/19	6/5	32/16	25/15	61/24
Fused						1/1	
Extra							
Missing		17/12	25/10	51/17	37/14	12/8	32/14
Other							
Ribs							
Incomplete oss.							
Fused/split		2/2		3/1	2/2		
Wavy			1/1				1/1
Less than 12							
More than 13		19/10	11/6	22/11	22/11	49/16	49/18
Other, extra							1/1
Vertebrae							
Incomplete oss.		2/2	2/2	6/2	4/4	11/3	1/1
Scrambled							
Fused							
Extra ctrs. oss.		23/14	12/8	3/3	4/4		8/3
Scoliosis		4/4	4/4	3/3			
Tail defects							
Other							
Skull							
Incomplete closure		1/1					
Missing							
Craniostosis			1/1				
Other; Occip./Parietals/ Facials; inc.		3/3	2/2		1/1	11/4	9/3
Extremities							
Incomplete oss.		36/14	55/17	64/14	73/18	36/13	78/1
Missing							
Extra							
Miscellaneous							
Pubis/Ilium/ischium; inc.		3/3	2/2				1/1
Hind leg left rotation			1/1				
Hyoid; missing			1/1		2/2		
Club foot						1/1	

* Numerator=Number of fetuses affected; Denominator=Number of litters affecte
** Positive control; 250 mg/kg.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 31 & 32; 37 through 40

Species Hamsters

Table 4

Average Body Weights *

Date March 31, 1972

Laboratory No. 0730 f

Group	Material	Dose Level	Day 0	6	8	10	14	**
		mg/kg	g					
31	Sham	0	97.4	103	104	116	137	(29)
32	Aspirin	250	99.7	104	105	117	135	(26)
37	FDA 71-5	40	97.0	101	104	115	137	(23)
38	FDA 71-5	100	102	105	108	119	142	(25)
39	FDA 71-5	240	101	106	108	117	136	(23)
40	FDA 71-5	600	98.9	103	104	115	137	(29)

* Of pregnant dams
** Number of surviving dams in parentheses (c.f. Table 1)

Appendix I

Teratology Study in Hamsters

Virgin adult female golden hamsters from an outbred strain were individually housed in mesh bottom cages in temperature and humidity controlled quarters with free access to food and fresh tap water at all times. They were mated (1 to 1) with mature males and the appearance of motile sperm in the vaginal smear was considered as Day 0 of gestation. Beginning on Day 6 and continuing daily through Day 10 of gestation, the indicated dose levels of the test material were administered by oral intubation; the controls were sham-treated.

Body weights were recorded on Days 0, 8, 10, and 14 of the gestation period. All animals were observed daily for appearance and behavior with particular attention to food consumption in order to better recognize any abnormalities resulting from anorexic effects in the pregnant animal.

On Day 14, all animals were subjected to Caesarian section under deep anesthesia and the numbers of implantation sites, resorption sites, live and dead fetuses were recorded. All live pups were weighed and the genital tract of each dam was examined for any anatomical abnormalities.

All fetuses were examined grossly for the presence of external congenital defects and one-third of each litter underwent detailed visceral examination under 10X magnification. The remaining two-thirds of the pups were cleared in potassium hydroxide, stained with alizarin red dye, and examined for the presence of sketal abnormalities.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 31 | Appendix II | Date March 31, 1972

Material Sham | Reproduction Data in Hamsters (Individual) | Laboratory No. 0730

Dose 0

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
S 0091	P	12	11	1	0	2.04	
S 0092	P	11	11	0	0	1.75	
S 0093	P	12	12	0	0	2.03	
S 0094	P	9	9	0	0	1.85	
S 0095	P	11	11	0	0	1.81	
S 0096	P	14	12	0	2	1.72	
S 0097	P	12	12	0	0	1.94	
S 0098	P	11	7	0	4	1.58	
S 0099	P	11	11	0	0	1.71	
S 0100	P	14	14	0	0	1.69	
S 0101	P	11	10	0	1	1.77	
S 0102	P	13	13	0	0	1.90	
S 0103	P	13	13	0	0	1.85	
S 0104	P	10	10	0	0	1.79	
S 0105	P	12	12	0	0	1.97	
S 0106	P	13	11	0	2	1.94	
S 0107	P	14	13	1	0	1.74	
S 0108	P	10	9	0	1	1.63	
S 0109	P	12	0	12	0	--	Died Day 14.
S 0110	P	12	9	0	3	1.91	
S 0111	P	11	9	1	1	2.16	
S 0112	P	11	11	0	0	1.64	
S 0113	P	11	11	0	0	1.84	
S 0114	P	12	12	0	0	1.64	
S 0115	P	8	8	0	0	2.20	
S 0116	P	12	11	0	1	1.82	
S 0117	P	14	14	0	0	1.82	
S 0118	P	13	13	0	0	2.11	
S 0119	P	11	11	0	0	2.03	
S 0120	P	12	12	0	0	2.11	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 32 | Appendix II | Date March 31, 1972

Material Aspirin | Reproduction Data in Hamsters (Individual) | Laboratory No. 0730

Dose 250 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
A 0091	P	9	9	0	0	1.78	
A 0092	P	14	14	0	0	1.88	
A 0093	P	11	11	0	0	1.76	
A 0094	P	12	10	0	2	2.08	
A 0095	NP	0				--	
A 0096	P	15	14	0	1	1.81	
A 0097	P	13	13	0	0	1.83	
A 0098	NP	0				--	
A 0099	P	13	11	0	2	1.82	
A 0100	P	10	10	0	0	1.90	
A 0101	P	12	12	0	0	1.56	
A 0102	P	15	14	0	1	1.83	
A 0103	P	16	15	0	1	1.50	
A 0104	P	9	8	0	1	1.97	
A 0105	P	11	11	0	0	1.96	
A 0106	P	19	18	0	1	1.57	
A 0107	P	13	11	0	2	1.82	
A 0108	P	12	12	0	0	1.60	
A 0109	P	9	0	0	9	--	
A 0110	P	14	14	0	0	1.65	
A 0111	P	13	13	0	0	1.70	
A 0112	P	15	0	15	0	--	Dam Died Day 8.
A 0113	NP	0				--	
A 0114	P	12	12	0	0	1.65	
A 0115	P	12	11	0	1	1.91	
A 0116	P	9	0	0	9	--	
A 0117	P	15	15	0	0	1.72	
A 0118	P	15	14	0	1	1.69	
A 0119	P	11	11	0	0	1.91	
A 0120	P	13	13	0	0	1.80	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 37 | Appendix II | Date March 31, 1972

Material FDA 71-5 | Reproduction Data in Hamsters (Individual) | Laboratory No. 0730 f

Dose 40 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
F 0001	P	13	13	0	0	1.86	
F 0002	P	11	11	0	0	1.89	
F 0003	NP	0				--	
F 0004	P	10	10	0	0	1.54	
F 0005	P	13	13	0	0	1.96	
F 0006	P	13	12	0	1	1.84	
F 0007	P	15	15	0	0	1.71	
F 0008	P	12	12	0	0	1.59	
F 0009	P	14	14	0	0	1.51	
F 0010	0	9	8	0	1	1.97	
F 0011	P	12	11	0	1	1.47	
F 0012	NP	0				--	
F 0013	P	13	10	0	3	1.69	
F 0014	P	12	12	0	0	1.94	
F 0015	P	14	14	0	0	1.77	
F 0016	P	12	10	0	2	2.05	
F 0017	P	12	12	0	0	2.00	
F 0018	NP	0				--	
F 0019	P	11	10	0	1	1.84	
F 0020	P	7	7	0	0	1.59	
F 0021	P	14	14	0	0	1.89	
F 0022	P	13	13	0	0	1.91	
F 0023	P	12	11	0	1	1.63	
F 0024	P	12	12	0	0	1.84	
F 0025	P	12	12	0	0	1.68	
F 0026	P	12	12	0	0	1.69	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 38 | Appendix II | Date March 31, 1972

Material FDA 71-5 | Reproduction Data in Hamsters (Individual) | Laboratory No. 0730 f

Dose 100 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
F 0031	P	11	10	0	1	1.93	
F 0032	P	14	14	0	0	1.73	
F 0033	P	11	11	0	0	1.92	
F 0034	P	11	11	0	0	1.94	
F 0035	P	12	12	0	0	1.65	
F 0036	P	15	15	0	0	1.68	
F 0037	P	12	12	0	0	1.70	
F 0038	P	13	13	0	0	1.84	
F 0039	P	13	13	0	0	1.71	
F 0040	P	13	10	0	3	1.72	
F 0041	P	17	17	0	0	1.90	
F 0042	P	12	11	0	1	1.55	
F 0043	NP	0				--	
F 0044	P	12	12	0	0	2.12	
F 0045	P	14	14	0	0	1.34	
F 0046	P	11	10	0	1	1.35	
F 0047	P	13	13	0	0	1.56	
F 0048	P	15	15	0	0	1.92	
F 0049	P	12	10	0	2	2.00	
F 0050	P	15	14	0	1	1.90	
F 0051	P	13	13	0	0	1.69	
F 0052	P	11	9	0	2	1.95	
F 0053	P	14	14	0	0	1.84	
F 0054	P	11	10	0	1	1.69	
F 0055	P	16	16	0	0	1.95	
F 0056	P	13	13	0	0	1.96	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 39 | Appendix II | Date March 31, 1972

Material FDA 71-5 | Reproduction Data in Hamsters (Individual) | Laboratory No. 0730 f

Dose 240 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
F 0061	P	11	10	0	1	1.83	
F 0062	P	14	14	0	0	1.82	
F 0063	P	13	13	0	0	1.81	
F 0064	P	13	13	0	0	1.92	
F 0065	P	15	15	0	0	1.90	
F 0066	P	12	12	0	0	1.93	
F 0067	P	14	13	0	1	1.81	
F 0068	P	10	10	0	0	1.97	
F 0069	P	15	15	0	0	1.67	
F 0070	NP	0				--	
F 0071	NP	0				--	
F 0072	P	14	14	0	0	1.72	
F 0073	P	13	12	1	0	1.93	
F 0074	P	13	13	0	0	1.77	
F 0075	P	14	14	0	0	1.87	
F 0076	P	13	13	0	0	1.95	
F 0077	P	13	13	0	0	1.98	
F 0078	P	14	12	0	2	1.71	
F 0079	P	13	0	13	0	--	Dam Died Day 8.
F 0080	P	10	0	0	10	--	
F 0081	P	13	12	0	1	1.67	
F 0082	P	12	12	0	0	1.72	
F 0083	P	13	0	13	0	--	
F 0084	P	10	9	0	1	1.63	
F 0085	P	10	10	0	0	1.66	
F 0086	P	11	11	0	0	2.02	
F 0087	NP	0				--	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 40 | Appendix II | Date March 31, 1972

Material FDA 71-5 | Reproduction Data in Hamsters (Individual) | Laboratory No. 0730 f

Dose 600 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
F 0091	P	11	11	0	0	1.83	
F 0092	P	6	4	0	2	1.90	
E 0093	P	13	13	0	0	1.86	
E 0094	P	14	14	0	0	1.56	
E 0095	P	12	12	0	0	1.88	
F 0096	P	14	13	0	1	1.76	
F 0097	P	14	13	0	1	1.73	
F 0098	P	13	12	0	1	1.73	
F 0099	P	14	13	0	1	1.74	
F 0100	P	6	0	0	6	--	
F 0101	P	14	14	0	0	1.57	
F 0102	P	12	10	0	2	1.57	
F 0103	P	15	15	0	0	1.71	
F 0104	P	11	11	0	0	1.70	
F 0105	P	13	13	0	0	1.66	
F 0106	P	11	11	0	0	2.01	
F 0107	P	12	11	0	1	2.05	
F 0108	P	15	13	0	2	1.85	
F 0109	P	10	10	0	0	2.00	
F 0110	P	11	11	0	0	2.33	Aborted Day 14.
F 0111	P	12	12	0	0	1.59	
F 0112	P	14	14	0	0	2.01	
F 0113	P	11	11	0	0	2.01	
F 0114	P	15	15	0	0	1.75	
F 0115	P	13	12	0	1	1.88	
F 0116	P	12	12	0	0	2.01	
F 0117	P	16	15	0	1	1.55	
F 0118	P	11	11	0	0	1.70	
F 0119	P	15	15	0	0	1.50	
F 0120	P	12	11	0	1	1.56	

* P = Pregnant; NP = Not Pregnant

Food and Drug Research Laboratories
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FINAL REPORT

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date May 1, 1972

Laboratory No. 0731 f
Contract No. FDA 71-260

Sample: Fine tan powdered material

Marking: FDA 71-5 (Calcium carragheenate)

Examination Requested: Teratologic evaluation of FDA 71- 5 in rabbits

Procedure: (See Appendix I)

Results: See Tables 1 through 4 and Appendix II

Conclusion: Attention is called to the fact that this is the fourth of a series of reports which will be issued in accordance with the terms of the contract cited above. Eventually, a total of at least 36 compounds will have been tested in 18 pairs; each pair being run concurrently against one sham-treated control and one positive control group. Because of the inherent variability of biological data of the type dealt with here, the accumulation and pooling of sequential sets of control values will greatly enhance the statistical value of the findings and the ultimate reliability of the test results.

For these reasons, the conclusion stated below is regarded as provisional and subject to reexamination in the light of later findings:

"The administration of up to 260 mg/kg (body weight) of the test material to pregnant rabbits for 13 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Kenneth Morgaridge

Kenneth Morgaridge, Ph.D.
Vice President

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups: 31 & 32; 37 through 40

Material: FDA 71-5

Date March 31, 1972

Laboratory No. 0731 f

Table 1

Fate Summary
(Rabbits)

Group	Material	Dose mg/kg	Total		At Term	
			Mated	Pregnant	Surviving (Total)	Number Pregnant
31	Sham	0	15	13	11	9
32	6-AN*	2.5	15	13	15	13
37	FDA 71-5	3.0	15	12	14	12
38	FDA 71-5	10	15	11	14	10
39	FDA 71-5	60	15	13	15	13
40	FDA 71-5	260	15	14	14	13

* Positive Control: 6-amino nicotinamide dosed on Day 9.

Group: 31 & 32; 37 through 40 FOOD AND DRUG RESEARCH LABORATORIES, INC. Date March 31, 1972

Material: FDA 71-5

Table 2
Reproduction Data
(Rabbits)

Laboratory No. 0731 f

Group:	31	32	37	38	39	40
Dose (mg/kg):	Sham	6-AN**	30	10	60	260
Pregnancies						
Total No.	13	13	12	15	13	14
Died or aborted (before Day 29)	4	0	1	2	0	1
To term (on Day 29)	9	13	12	10	13	13
Corpora lutea						
Total No.	243	235	207	201	172	187
Average/dam mated	16.2	15.7	13.8	13.4	11.5	12.5
Live litters						
Total No.*	9	10	10	9	12	9
Implant sites						
Total No. (at term)	39	59	59	51	70	56
Average/dam*	4.33	4.54	4.92	5.10	5.38	4.3
Resorptions						
Total No.*	5	15	9	13	6	7
Dams with 1 or more sites resorbed	3	8	5	4	6	5
Dams with all sites resorbed	0	2	2	1	0	4
Per cent partial resorptions	33.3	61.5	41.7	40.0	46.2	38.5
Per cent complete resorptions	--	15.4	16.7	10.0	--	30.8
Live fetuses						
Total No. (at term)	34	43	50	38	64	49
Average/dam*	3.78	3.31	4.17	3.80	4.92	3.0
Dead fetuses						
Total No.*	0	1	0	0	0	0
Dams with 1 or more dead	-	1	-	-	-	-
Dams with all dead	-	0	-	-	-	-
Per cent partial dead	-	-	-	-	-	-
per cent all dead	-	7.69	-	-	-	-
Average fetus weight, g	37.3	31.0	42.0	38.9	37.2	37.1

* Includes only those dams examined at term.

** Positive control: 2.5 mg/kg 6-amino nicotinamide dosed on Day 9.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 31 & 32; 37 through 40 Laboratory No. 0731 f

Table 3

Material FDA 71-5 Date March 31, 1972

Summary of Skeletal Findings *

(Rabbits)

Findings	Group No.	31	32	37	38	39	40
	Dose (mg/kg)	Sham	6-AN**	3	10	60	260
Live Fetuses Examined		32/9	42/10	50/10	33/9	64/12	57/1
Sternebrae							
Incomplete oss.		7/5	16/7	8/7	7/4	15/6	16/8
Scrambled							
Bipartite			8/6		2/1		
Fused		2/1	12/6		1/1	1/1	1/1
Extra			3/3	5/5	2/2	4/2	1/1
Missing			1/1	1/1	1/1		1/1
Other							
Ribs							
Incomplete oss.			2/2				
Fused/split			7/6		2/1		
Wavy							
Less than 12				4/1	1/1	1/1	1/1
More than 13		1/1	1/1		3/3	9/4	12/4
Other							
Vertebrae							
Incomplete oss.							
Scrambled			1/1				
Fused							
Extra ctrs. oss.							
Scoliosis			1/1				
Tail defects			23/7	2/1			
Other; Scrambled tail					1/1		
Skull							
Incomplete closure						1/1	
Missing							
Craniostosis		1/1	8/2	3/3	6/4	7/3	9/4
Other: Eyes; incomplete			8/2				
Extremities							
Incomplete oss.							
Missing							
Extra							
Miscellaneous							
Club feet			5/3				

* Numerator=Number of fetuses affected; Denominator=Number of litters affecte
** Positive control: 6- amino nicotinamide dosed on Day 9.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 31 & 32; 37 through 40 Date March 31, 1972

Material FDA 71-5 Laboratory No. 0731 f

Table 3-a

Summary of Soft Tissue Abnormalities

(Rabbits)

Group	Material	Dose level mg/kg	Dam	Number of Pups	Description
31	Sham	0	S-1051	2	Euryopia
				1	Hair lip
32	6-AN*	2.5	Z-1046	5	Anopia
				3	Club feet
32	6-AN		Z-1047	7	Anopia
				3	Cleft palate
				2	Hair lip
32	6-AN		Z-1049	6	Anopia
				4	Club feet
				3	Cleft palate
				3	Hair lip
32	6-AN		Z-1052	1	Anopia
				2	Missing digits
				1	Dysgnathia
32	6-AN		Z-1053	2	Anopia
				3	Club feet
32	6-AN		Z-1054	3	Club feet
				1	Anopia
				1	Hair lip
32	6-AN		Z-1055	7	Anopia
				5	Club feet
32	6-AN		Z-1059	5	Anopia
				3	Club feet
				3	Cleft palate

* 6 - amino nicotinamide dosed on Day 9.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 31 & 32; 37 through 40

Species Rabbits

Table 4

Average Body Weights

Date March 31, 1972

Laboratory No. 0731 f

Group	Material	Dose Level	Day 0	6	12	18	29 **
		mg/kg	kg				
31	Sham	0	2.34	2.35	2.38	2.36	2.41 (9)
32	6-AN***	2.5	2.21	2.18	2.27	2.27	2.36 (13)
37	FDA 71-5	3.0	2.35	2.39	2.44	2.51	2.55 (12)
38	FDA 71-5	10	2.50	2.57	2.54	2.66	2.63 (10)
39	FDA 71-5	60	2.25	2.28	2.32	2.37	2.47 (13)
40	FDA 71-5	260	2.28	2.28	2.28	2.29	2.42 (13)

* Of pregnant dams.
** Number of surviving dams in parentheses (c.f. Table 1)
*** Positive control: 6-amino nicotinamide dosed on Day 9.

Appendix I

Teratology Study in Rabbits

Virgin, adult, Dutch-belted female rabbits were individually housed in mesh bottom cages in temperature and humidity-controlled quarters with free access to food and fresh tap water. On Day 0, each doe was given an injection of 0.4 ml of human chorionic gonadotropin (400 IU) via the marginal ear vein. Three hours later, each doe was inseminated artificially with 0.3 ml of diluted semen from a proven donor buck using approximately 20×10^6 motile sperm according to the procedure described by Vogin et al (Pharmacologist 11, 282 (1969)). Beginning on Day 6 and continuing daily through Day 18 the females were dosed with the indicated dosages by oral intubation; the controls were sham treated.

Body weights were recorded on Days 0, 6, 12, 18, and 29 of gestation. All animals were observed daily for appearance and behavior, with particular attention to food consumption and body weight in order to rule out any abnormalities which may have occurred as a result of anorexic effects in the pregnant female animal.

On Day 29 all does were subjected to Caesarean section under surgical anesthesia, and the numbers of corpora lutea, implantation sites, resorption sites and live and dead fetuses were recorded. Body weights of the live pups were also recorded. The urogenital tract of each animal was examined in detail for normality. In addition all fetuses underwent a detailed gross examination for the presence of external congenital abnormalities. The live fetuses of

each litter were then placed in an incubator for 24 hours for the evaluation of neonatal survival. All surviving pups were sacrificed, and all pups examined for visceral abnormalities (by dissection). All fetuses were then cleared in potassium hydroxide (KOH), stained with alizarin red S dye and examined for skeletal defects.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 31 | Appendix II | Date March 31, 1972

Material Sham | Reproduction Data in Rabbits (Individual) | Laboratory No. 0731

Dose 0

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
S 1046	P	13	4		4		----	Died Day 14
S 1047	P	19	1	1			36.4	
S 1048	NP	12	0				----	
S 1049	P	19	5	5			36.9	
S 1050	P	25	5	3		2	43.6	
S 1051	P	15	6	5		1	17.7	
S 1052	P	8	6	6			42.7	
S 1053	P	16	6	6			32.8	
S 1054	P	20	3	3			38.4	
S 1055	P	19	1	1			46.1	
S 1056	NP	2	0				----	
S 1057	P	13	4		4		----	Died Day 10
S 1058	P	23	6	4		2	40.7	
S 1059	P	20	5		5		----	Died Day 10
S 1060	P	19	2		2		----	Died Day 11

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 32 — Appendix II — Date March 31, 1972

Material 6 - AN — Reproduction Data in Rabbits (Individual) — Laboratory No. 0731

Dose 2.5 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
Z 1046	P	21	6	6			28.8	
Z 1047	P	18	7	7			27.0	
Z 1048	NP	8	0				----	
Z 1049	P	21	6	5		1	29.2	
Z 1050	P	11	1			1	----	
Z 1051	P	8	2			2	----	
Z 1052	P	9	4	1		3	21.6	
Z 1053	P	11	4	3		1	29.7	
Z 1054	P	39	6	3		3	29.0	
Z 1055	P	25	8	7	1		27.6	
Z 1056	P	15	5	4		1	34.0	
Z 1057	P	8	2	2			43.0	
Z 1058	P	14	3			3	----	
Z 1059	P	23	5	5			40.3	
Z 1060	NP	4	0				----	

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 37 — Appendix II — Date March 31, 1972

Material FDA 71-5 — Reproduction Data in Rabbits (Individual) — Laboratory No. 0731 f

Dose 3 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
F 1001	P	20	7	7			30.4	
F 1002	P	16	5	4		1	45.6	
F 1003	P	11	4	1		3	49.0	
F 1004	NP	9	0				----	
F 1005	P	22	9	9			38.8	
F 1006	NP	1	0				----	
F 1007	P	4	2	0		2	----	
F 1008	P	14	4	4			43.4	
F 1009	NP	11	0				----	Died Day 23.
F 1010	P	33	6	6			34.1	
F 1011	P	12	2	2			51.3	
F 1012	P	18	2			2	----	
F 1013	P	6	3	2		1	54.1	
F 1014	P	8	4	4			37.2	
F 1015	P	22	11	11			36.9	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEAR) LABORATORIES, INC.

Group 38 | Appendix II | Date March 31, 1972

Material FDA 71-5 | Reproduction Data in Rabbits (Individual) | Laboratory No. 0731 f

Dose 10 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
F 1016	P	25	7	7			36.2	
F 1017	P	23	1	1			39.6	
F 1018	NP	7	0				----	
F 1019	NP	3	0				----	
F 1020	P	30	2	2			39.0	
F 1021	P	22	10	8		2	38.5	
F 1022	NP	0	0				----	
F 1023	P	9	8			8	----	
F 1024	P	19	7	7			39.4	
F 1025	P	13	2	2			42.7	
F 1026	P	10	4	4			43.2	
F 1027	P	11	6	4		2	35.3	
F 1028	P	13	4	3		1	36.8	
F 1029	NP	0	0				----	
F 1030	P	16	8		8		----	Died Day 25.

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEAI .1 LABORATORIES, INC.

Group 39 | Appendix II | Date March 31, 1972

Material FDA 71-5 | Reproduction Data in Rabbits (Individual) | Laboratory No. 0731 f

Dose 60 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead			
F 1031	P	25	7	6		1	39.5	
F 1032	P	4	1			1	----	
F 1033	P	20	9	8		1	37.6	
F 1034	P	11	4	4			36.9	
F 1035	P	27	8	8			32.9	
F 1036	P	6	6	6			38.5	
F 1037	P	8	5	5			36.5	
F 1038	NP	3	0				----	
F 1039	P	8	4	3		1	38.7	
F 1040	P	13	8	7		1	39.6	
F 1041	P	11	4	3		1	37.0	
F 1042	P	9	5	5			28.0	
F 1043	P	11	6	6			38.1	
F 1044	NP	2	0				----	
F 1045	P	14	3	3			44.2	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 40 — Appendix II — Date March 31, 1972

Material FDA 71-5 — Reproduction Data in Rabbits (Individual) — Laboratory No. 0731 f

Dose 260 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
F 1046	NP	7	0				----	
F 1047	P	31	4	4			39.9	
F 1048	P	30	8	8			36.7	
F 1049	P	11	1			1	----	
F 1050	P	14	5	5			33.4	
F 1051	P	15	7	7			33.7	
F 1052	P	11	3	3			42.6	
F 1053	P	6	3			3	----	
F 1054	P	7	4	4			----	Aborted Day 20
F 1055	P	7	6	6			37.2	
F 1056	P	5	1			1	----	
F 1057	P	9	5	5			36.4	
F 1058	P	7	1			1	----	
F 1059	P	14	8	7		1	34.3	
F 1060	P	13	4	4			40.3	

* P = Pregnant; NP = Not Pregnant

Food and Drug Research Laboratories
INCORPORATED

Maurice Avenue at 58th Street
Maspeth, New York 11378
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DRAFT REPORT

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date March 31, 1972

Laboratory No. 0728 d
Contract No. FDA 71-260

Sample: Fine light tan powdered material

Marking: FDA 71-3 (Sodium carragheenate)

Examination Requested: Teratologic evaluation of FDA 71- 3 in mice.

Procedure: See Appendix I

Results: See Tables 1 through 4 and Appendix II

Conclusion: Subject to reexamination in the light of later findings, the following is concluded:

"The administration of the test material in graded dosage levels up to 900 mg/kg (body weight) to pregnant mice for 10 consecutive days caused an apparent increase in the number of resorptions and/or fetal deaths in utero. There was a corresponding decrease in the number of live pups and a reduction in pup weight at delivery, both of which appear to have been dose-dependent. A concurrent retardation in skeletal maturation may be inferred from the increased incidence of missing sternebrae and incomplete skull closure. All other soft and skeletal tissue abnormalities were probably within the accepted limits of variability for the species."

It was concluded that the test material was fetotoxic in the pregnant mouse without exhibiting frank teratogenicity.

(See following page for comment)

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Kenneth Morgareidge

Kenneth Morgareidge, Ph.D.
Vice President

Comment: Attention is called to the fact that this is the fourth of a series of reports which will be issued in accordance with the terms of the contract cited above. Eventually, a total of at least 36 compounds will have been tested in 18 pairs; each pair being run concurrently against one sham-treated control and one positive control group. Because of the inherent variability of biological data of the type dealt with here, the accumulation and pooling of sequential sets of control values will greatly enhance the statistical value of the findings and the ultimate reliability of the test results.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups: 31 through 36

Material: FDA 71-3

Date March 31, 1972

Laboratory No. 0728 d

Table 1

Fate Summary
(Mice)

Group	Material	Dose mg/kg	Total		At Term	
			Mated	Pregnant	Surviving (Total)	Number Pregnant
31	Sham	0	27	27	26	26
32	Aspirin*	150	26	24	22	21
33	FDA 71-3	10	24	22	23	21
34	FDA 71-3	45	27	26	27	26
35	FDA 71-3	470	29	24	24	26
36	FDA 71-3	900	40	25	32	19

* Positive Control

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group: 31 through 36

Material: FDA 71-3

Table 2
Reproduction Data
(Mice)

Date March 31, 1972

Laboratory No. 0728 d

Group:	31	32	33	34	35	36
Dose (mg/kg):	Sham	Aspirin**	10	45	470	900
Pregnancies						
Total No.	27	24	22	26	27	25
Died or Aborted (before Day 17)	1	4	1	0	2	9
To term (on Day 17)	26	21	21	26	26	19
Live litters						
Total No. *	26	20	21	26	25	16
Implant sites						
Total No.	306	241	248	295	315	232
Average/dam *	11.8	11.5	11.8	11.3	12.1	12.2
Resorptions						
Total No. *	10	21	13	6	21	59
Dams with 1 or more sites resorbed	4	7	7	6	8	12
Dams with all sites resorbed	0	1	0	0	0	2
Per cent partial resorptions	15.4	33.3	33.3	23.1	30.8	63.2
Per cent complete resorptions	-	4.76	-	-	-	10.5
Live fetuses						
Total No.	296	218	232	288	282	167
Average/dam *	11.4	10.4	11.0	11.1	10.8	8.79
Dead fetuses						
Total No. *	0	2	3	1	12	6
Dams with 1 or more dead	-	2	2	1	5	3
Dams with all dead	-	0	0	0	0	0
Per cent partial dead	-	9.52	9.52	3.85	19.2	15.8
Per cent all dead	-	-	-	-	-	-
Average fetus weight, g	0.96	0.92	0.97	0.99	0.89	0.83

* Includes only those dams examined at term.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 31 through 36 | Table 3 | Laboratory No. 0728 d

Material FDA 71-3 | Date March 31, 1972

Summary of Skeletal Findings *

(Mice)

Findings	Group No.	31	32	33	34	35	36
	Dose (mg/kg)	Sham	Aspirin**	10	45	470	900
Live Fetuses Examined		193/25	151/20	162/21	199/26	195/25	107/
Sternebrae							
Incomplete oss.		23/13	44/16	24/12	17/12	61/18	20/
Scrambled							1/
Bipartite		5/3	11/7	9/6	6/6	22/9	1/
Fused							1/
Extra							
Missing		4/3	2/2	4/2	4/4	20/6	19/
Ribs							
Incomplete oss.							
Fused/split							
Wavy					1/1		
Less than 12							
More than 13		16/9	7/5	13/9	24/13	9/6	4/
Other							
Vertebrae							
Incomplete oss.					1/1		
Scrambled							
Fused							
Extra ctrs. oss.		1/1					
Scoliosis							
Tail defects							
Other							
Skull							
Incomplete closure			2/1	3/2	2/2	9/8	10/
Missing							
Craniostosis							
Other; exencephaly		1/1				1/1	
Extremities							
Incomplete oss.				2/2		1/1	2/
Missing							
Extra							
Miscellaneous							
Hyoid; reduced		8/5	14/8	15/9	22/13	18/9	5/
Hyoid; missing		8/7	19/9	14/7	11/8	13/11	16/

* Numerator=Number of fetuses affected; Denominator=Number of litters affecte

** Positive control : 150 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 31 through 36 Date March 31, 1972

Material FDA 71-3 Laboratory No. 0728 d

Table 3-a

Summary of Soft Tissue Abnormalities

(Mice)

Group	Material	Dose level mg/kg	Dam	Number of Pups	Description
32	Aspirin	150	A-8104	1	Fetal monster
				1	Anopia
				1	Mouth and nasal absent

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 31 through 36

Species Mice

Table 4

Average Body Weights *

Date March 31, 1972

Laboratory No. 0728 d

Group	Material	Dose Level	Day 0	6	11	15	17	**
		mg/kg	g					
31	Sham	0	26.6	29.8	34.0	40.2	48.1	(26)
32	Aspirin	150	26.8	30.0	33.0	37.9	43.6	(21)
33	FDA 71-3	10	27.9	30.3	34.3	43.5	48.1	(21)
34	FDA 71-3	45	26.3	29.6	33.8	42.6	47.9	(26)
35	FDA 71-3	470	27.2	29.2	31.2	39.3	46.2	(26)
36	FDA 71-3	900	27.1	29.3	30.6	35.4	39.5	(19)

* Of pregnant dams
** Number of surviving dams in parentheses (c.f. Table 1)

Appendix I

Teratology Study in Mice

Virgin adult female albino CD-1 outbred mice were individually housed in disposable plastic cages in temperature and humidity-controlled quarters with free access to food and fresh tap water. They were mated with young adult males, and observation of the vaginal sperm plug was considered Day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, the females were dosed with the indicated dosages by oral intubation; the controls were sham treated.

Body weights were recorded on Days 0, 6, 11, 15, and 17 of gestation. All animals were observed daily for appearance and behavior with particular attention to food consumption and weight, in order to rule out any abnormalities which may have occurred as a result of anorexic effects in the pregnant female animal.

On Day 17 all dams were subjected to Caesarean section under surgical anesthesia, and the numbers of implantation sites, resorption sites, and live and dead fetuses were recorded. The body weights of the live pups were also recorded. The urogenital tract of each dam was examined in detail for anatomical normality.

All fetuses were examined grossly for the presence of external congenital abnormalities. One-third of the fetuses of each litter underwent detailed visceral examinations employing 10X magnification. The remaining two-thirds were cleared in potassium hydroxide (KOH), stained with alizarin red S dye and examined for skeletal defects.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 31 | Appendix II | Date March 31, 1972

Material Sham | Reproduction Data in Mice (Individual) | Laboratory No. 0728

Dose 0

Dam No.	Fate *	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
S 8091	P	11	9		2	0.92	
S 8092	P	14		14		----	Died Day 15
S 8093	P	14	14			0.97	
S 8094	P	13	10		3	0.94	
S 8095	P	13	13			0.85	
S 8096	P	14	14			0.93	
S 8097	P	16	16			0.95	
S 8098	P	11	11			0.96	
S 8099	P	13	13			0.97	
S 8100	P	13	13			0.90	
S 8101	P	8	8			0.99	
S 8102	P	12	12			1.11	
S 8103	P	8	8			0.97	
S 8104	P	11	11			0.91	
S 8105	P	10	10			0.95	
S 8106	P	13	13			0.89	
S 8107	P	13	13			0.88	
S 8108	P	11	11			0.92	
S 8109	P	13	12		1	0.99	
S 8110	P	15	15			1.04	
S 8111	P	15	15			1.06	
S 8112	P	9	9			1.02	
S 8113	P	12	12			0.98	
S 8114	P	3	3			1.08	
S 8115	P	13	13			0.87	
S 8116	P	12	8		4	1.04	
S 8117	P	10	10			0.87	

* P= Pregnant; NP= Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 32 | Appendix II | Date March 31, 1972

Material Aspirin | Reproduction Data in Mice (Individual) | Laboratory No. 0728

Dose 150 mg/kg

Dam No.	Fate *	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
A 8091	P	14		13	1	----	Died Day 16
A 8092	P	15		14	1	----	Died Day 16
A 8093	P	12	12			0.69	
A 8094	P	11	11			0.96	
A 8095	P	15			15	----	
A 8096	P	11	10		1	0.95	
A 8097	P	13	12		1	0.91	
A 8098	P	10	10			0.84	
A 8099	P	12	12			0.85	
A 8100	P	14	14			0.85	
A 8101	P	10	9		1	1.22	
A 8102	P	10	10			1.02	
A 8103	P	15	14	1		0.89	
A 8104	P	10	9	1		0.90	
A 8105	NP	0				----	Died Day 13
A 8106	--	--				----	Number not assigned.
A 8107	P	11	10		1	0.88	
A 8108	P	12	12			0.84	
A 8109	P	12	12			1.01	
S 8110	P	12		12		----	Died Day 14
S 8111	P	11	10		1	0.96	
S 8112	P	8	8			0.91	
S 8113	P	10	10			0.92	
S 8114	P	12	12			1.00	
S 8115	P	9	8		1	0.93	
S 8116	NP	0				----	
S 8117	P	13	13			0.93	

* P= Pregnant; NP= Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 33 | Appendix II | Date March 31, 1972

Material FDA 71-3 | Reproduction Data in Mice (Individual) | Laboratory No. 0728 d

Dose 10 mg/kg

Dam No.	Fate *	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
D 8001	NP	0				----	
D 8002	P	13	13			0.82	
D 8003	P	13	11	2		0.86	
D 8004	NP	0				----	
D 8005	P	12	10		2	0.97	
D 8006	P	12	12			0.96	
D 8007	P	16	15		1	1.01	
D 8008	P	11	11			0.95	
D 8009	P	9		9		----	Died Day 15
D 8010	P	8	7		1	0.99	
D 8011	P	12	12			1.02	
D 8012	P	11	11			1.06	
D 8013	P	11	10		1	0.82	
D 8014	P	11	6		5	0.87	
D 8015	P	9	9			0.84	
D 8016	P	12	12			0.90	
D 8017	P	12	11	1		0.86	
D 8018	P	14	14			0.96	
D 8019	P	12	12			1.10	
D 8020	P	12	12			0.98	
D 8021	P	10	10			1.37	
D 8022	P	13	11		2	1.03	
D 8023	P	12	12			0.91	
D 8024	P	12	11		1	1.06	

* P= Pregnant; NP= Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 34 | Appendix II | Date March 31, 1972

Material FDA 71-3 | Reproduction Data in Mice (Individual) | Laboratory No. 0728 d

Dose 45 mg/kg

Dam No.	Fate *	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
D 8031	P	9	9			0.80	
D 8032	P	16	16			0.90	
D 8033	P	10	10			0.96	
D 8034	P	9	9			1.02	
D 8035	P	7	7			1.07	
D 8036	P	14	14			0.92	
D 8037	P	8	8			0.96	
D 8038	P	11	11			1.03	
D 8039	P	9	9			1.01	
D 8040	P	11	10		1	0.89	
D 8041	P	8	8			0.92	
D 8042	P	11	10		1	1.15	
D 8043	P	12	12			1.26	
D 8044	P	11	10		1	0.90	
D 8045	P	15	15			0.98	
D 8046	P	13	12		1	0.94	
D 8047	P	13	12		1	0.99	
D 8048	P	14	14			1.04	
D 8049	P	8	8			1.07	
D 8050	P	12	12			0.92	
D 8051	P	10	10			1.00	
D 8052	P	9	9			1.03	
D 8053	P	16	16			0.87	
D 8054	P	13	13			1.03	
D 8055	P	12	11	1		0.92	
D 8056	P	13	12		1	1.22	
D 8057	NP	0				----	

* P= Pregnant; NP= Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 35 | Appendix II | Date March 31, 1972

Material FDA 71-3 | Reproduction Data in Mice (Individual) | Laboratory No. 0728 d

Dose 470 mg/kg

Dam No.	Fate *	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
D 8061	P	13	13			0.97	
D 8062	P	20	20			0.96	
D 8063	P	15		15		----	Died Day 17
D 8064	P	11	11			0.75	
D 8065	P	12	12			0.76	
D 8066	P	10	9	1		0.68	
D 8067	-	--				----	Number not assigned.
D 8068	NP	-				----	Died Day 15
D 8069	P	11		7	4	----	
D 8070	P	12	12			0.80	
D 8071	P	11	10	1		0.89	
D 8072	P	12	12			0.86	
D 8073	P	10	6		4	0.85	
D 8074	P	10	10			0.94	
D 8075	P	13	13			0.98	
D 8076	P	14	14			0.96	
D 8077	-	-				----	Number not assigned.
D 8078	P	11	10		1	0.92	
D 8079	P	11	10	1		0.88	
D 8080	P	10	10			0.98	
D 8081	P	13	13			0.98	
D 8082	P	10	10			1.12	
D 8083	NP	-				----	
D 8084	P	12	7		5	0.81	
D 8085	P	14	11		3	0.89	
D 8086	P	12	11		1	0.81	
D 8087	P	15	13	2		1.04	
D 8088	P	11	11			0.86	
D 8089	P	12	11		1	0.76	
D 8090	P	13	11		2	0.89	
D 8134	P	12	12			0.83	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 36 | Appendix II | Date March 31, 1972

Material FDA 71-3 | Reproduction Data in Mice (Individual) | Laboratory No. 0728 d

Dose 900 mg/kg

Dam No.	Fate *	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
D 8091	P	15			15	----	
D 8092	-	--				----	Number not assigned.
D 8093	P	12			12	----	Died Day 10
D 8094	P	10	8		2	0.74	
D 8095	-	--				----	Number not assinged.
D 8096	P	11	11			0.97	
D 8097	NP	--				----	
D 8098	P	12	12			0.86	
D 8099	P	12			12	----	Died Day 9
D 8100	P	12		3	9	----	
D 8101	P	12			12	----	
D 8102	NP	--				----	
D 8103	NP	--				----	
D 8104	P	13	8		5	0.99	
D 8105	NP	--				----	
D 8106	NP	--				----	
D 8107	NP	--				----	
D 8108	NP	--				----	Died Day 10
D 8109	P	12			12	----	Died Day 9
D 8110	NP	--				----	
D 8111	P	8	8			0.81	
D 8112	NP	--				----	
D 8113	P	14	14			1.03	
D 8114	NP	--				----	
D 8115	P	13	12	1		1.01	
D 8116	NP	--				----	Died Day 15
D 8117	P	13	10	2	1	0.85	
D 8118	P	14	13		1	1.11	
D 8119	-	--				----	Number not assigned.

* P= Pregnant; NP= Not Pregnant

(continued on following page)

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 36 (concluded) Appendix II Date March 31, 1972

Material FDA 71-3 Reproduction Data in Mice (Individual) Laboratory No. 0728 d

Dose 900 mg/kg

Dam No.	Fate *	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
D 8120	NP	--				----	Died Day 17
D 8121	P	13	11		2	0.57	
D 8122	P	8	8			----	Died Day 8
D 8123	NP					----	
D 8124	P	11	8		3	0.71	
D 8125	P	11	5		6	0.52	
D 8126	P	12	12			0.82	
D 8127	P	11	11			----	Died Day 8
D 8128	P	13	13			----	Died Day 12
D 8129	P	15	14		1	0.66	
D 8130	NP	--				----	
D 8131	NP	--				----	
D 8132	P	9	7		2	0.70	
D 8133	P	14	14			0.97	

* P= Pregnant; NP= Not Pregnant

Food and Drug Research Laboratories
INCORPORATED

Maurice Avenue at 58th Street
Maspeth, New York 11378
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DRAFT REPORT

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date March 31, 1972

Laboratory No. 0729 d
Contract No. FDA 71-260

Sample: Fine light tan powdered material.

Marking: FDA 71-3 (Sodium carragheenate)

Examination Requested: Teratologic evaluation of FDA 71- 3 in rats

Procedure: See Appendix I

Results: See Tables 1 through 4 and Appendix II

Conclusion: Subject to reexamination in the light of later findings, the following is concluded:

"The administration of the test material in graded dosage levels up to 600 mg/kg (body weight) to pregnant rats for 10 consecutive days caused an apparent increase in the number of resorption sites observed with or without a corresponding decrease in the number of live pups delivered. At the highest dose level, there may have been a decrease in the birth weight of the pups. A concurrent retardation in skeletal maturation was indicated by a dose-dependent increase in missing sternebrae. There were no other findings in either soft or skeletal tissues which appeared to be treatment-related.

It was concluded that the test material depressed fetal development in the pregnant rat and caused an increase in early fetal deaths (resorptions). There was no evidence of frank teratogenicity.

(See following page for comment)

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Kenneth Margareidge, Ph.D.
Vice President

Comment: Attention is called to the fact that this is the fourth of a series of reports which will be issued in accordance with the terms of the contract cited above. Eventually, a total of at least 36 compounds will have been tested in 18 pairs; each pair being run concurrently against one sham-treated control and one positive control group. Because of the inherent variability of biological data of the type dealt with here, the accumulation and pooling of sequential sets of control values will greatly enhance the statistical value of the findings and the ultimate reliability of the test results.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups: 31 through 36

Material: FDA 71-3

Date March 31, 1972

Laboratory No. 0729 d

Table 1

Fate Summary
(Rats)

Group	Material	Dose mg/kg	Total		At Term	
			Mated	Pregnant	Surviving (Total)	Number Pregnant
31	Sham	0	25	23	25	23
32	Aspirin*	250	29	27	25	24
33	FDA 71-3	40	24	23	23	23
34	FDA 71-3	100	25	24	23	23
35	FDA 71-3	240	25	21	24	20
36	FDA 71-3	600	30	26	29	25

* Positive Control

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group: 31 through 36

Material: FDA 71-3

Date March 31, 1972

Laboratory No. 0729 d

Table 2
Reproduction Data
(Rats)

Group:	31	32	33	34	35	36
Dose (mg/kg):	Sham	Aspirin**	40	100	240	600
Pregnancies						
Total No.	23	27	23	24	21	26
Died or Aborted (before Day 20)	0	4	1	2	1	1
To term (on Day 20)	23	24	23	23	20	25
Live litters						
Total No. *	23	18	23	23	20	25
Implant sites						
Total No.	252	267	240	229	236	275
Average/dam *	11.0	11.1	10.4	9.96	11.8	11.0
Resorptions						
Total No. *	7	110	7	10	27	26
Dams with 1 or more sites resorbed	5	15	7	6	8	9
Dams with all sites resorbed	0	5	0	0	1	0
Per cent partial resorptions	21.7	62.5	30.4	26.1	40.0	36.0
Per cent complete resorptions	--	20.8	--	--	5.00	--
Live fetuses						
Total No.	245	156	233	219	209	250
Average/dam *	10.7	6.50	10.1	9.52	10.5	10.0
Dead fetuses						
Total No. *	0	1	0	0	0	0
Dams with 1 or more dead	-	1	-	-	-	-
Dams with all dead	-	0	-	-	-	-
Per cent partial dead	-	4.17	-	-	-	-
Per cent all dead	-	0	-	-	-	-
Average fetus weight, g	3.75	2.47	3.97	3.92	3.74	3.16

* Includes only those dams examined at term.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 31 through 36 | Table 3 | Laboratory No. 0729 d

Material FDA 71-3 | Date March 31, 1972

Summary of Skeletal Findings *

(Rats)

Findings	Group No.	31	32	33	34	35	36
	Dose (mg/kg)	Sham	Aspirin**	40	100	240	600
Live Fetuses Examined		171/23	103/17	162/23	155/23	144/20	173/
Sternebrae							
Incomplete oss.		93/23	41/15	86/21	60/21	71/20	103/
Scrambled		18/11	16/6	16/10	9/7	10/9	24/
Bipartite		16/10	27/10	11/8	7/6	15/9	10/
Fused							
Extra							1/
Missing		23/10	91/17	22/9	38/12	59/15	81/
Other							
Ribs							
Incomplete oss.			2/2				
Fused/split			3/3				
Wavy		1/1	18/8	3/2	3/3	2/2	
Less than 12							
More than 13		2/2	19/7	5/2	6/4	1/1	
Other							
Vertebrae							
Incomplete oss.			9/5	1/1			
Scrambled			15/7				
Fused							
Extra ctrs. oss.							
Scoliosis			1/1				
Tail defects							
Other							
Skull							
Incomplete closure		19/12	10/3	46/16	45/16	34/14	13/
Missing							
Craniostosis		2/1		4/2			
Other							
Extremities							
Incomplete oss.			1/1				
Missing			1/1				
Extra							
Miscellaneous							
Hyoid; reduced		12/7	28/6	19/11	5/4	9/7	7/
Hyoid; missing		11/6	51/13	13/7	15/8	23/12	31/

* Numerator=Number of fetuses affected; Denominator=Number of litters affect
** Positive control; 250 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 31 through 36 Date March 31, 1972

Material FDA 71-3 Laboratory No. 0729 d

Table 3-a

Summary of Soft Tissue Abnormalities

(Rats)

Group	Material	Dose level mg/kg	Dam	Number of Pups	Description
32	Aspirin	250	A-9091	1	Meningoencephalocele
32	Aspirin	250	A-9104	6	Anopia
				5	Club feet
				6	Hydrocephalus
				6	Umbilical hernia
				5	Cleft palate
				6	Meningoencephalocele

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 31 through 36

Species Rats

Table 4

Average Body Weights *

Date March 31, 1972

Laboratory No. 0729 d

Group	Material	Dose Level	Day 0	6	11	15	20	**
		mg/kg	g					
31	Sham	0	216	236	250	270	322	(23)
32	Aspirin	250	230	247	254	269	308	(24)
33	FDA 71-3	40	207	226	239	256	315	(23)
34	FDA 71-3	100	209	226	237	252	308	(23)
35	FDA 71-3	240	209	233	243	255	320	(20)
36	FDA 71-3	600	218	236	239	251	308	(25)

* Of pregnant dams

** Number of surviving dams in parentheses (c.f. Table 1)

Appendix I

Teratology Study in Rats

Virgin adult female albino rats (Wistar derived stock) were individually housed in mesh bottom cages in temperature and humidity-controlled quarters with free access to food and fresh tap water. They were mated with young adult males, and observation of the vaginal sperm plug was considered Day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, the females were dosed with the indicated dosages by oral intubation; the controls were sham treated.

Body weights were recorded on Days 0, 6, 11, 15, and 20 of gestation. All animals were observed daily for appearance and behavior with particular attention to food consumption and weight, in order to rule out any abnormalities which may have occurred as a result of anorexic effects in the pregnant female animal.

On Day 20 all dams were subjected to Caesarean section under surgical anesthesia, and the numbers of implantation sites, resorption sites, and live and dead fetuses were recorded. The body weights of the live pups were also recorded. The urogenital tract of each dam was examined in detail for anatomical normality.

All fetuses were examined grossly for the presence of external congenital abnormalities. One-third of the fetuses of each litter underwent detailed visceral examinations employing 10X magnification. The remaining two-thirds were cleared in potassium hydroxide (KOH), stained with alizarin red S dye and examined for skeletal defects.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 31 | Appendix II | Date March 31, 1972

Material Sham | Reproduction Data in Rats (Individual) | Laboratory No. 0729

Dose 0

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
S 9091	P	11	11			4.09	
S 9092	P	11	11			3.84	
S 9093	P	9	9			4.13	
S 9094	P	12	12			3.70	
S 9095	P	12	12			3.36	
S 9096	P	11	11			3.64	
S 9097	P	6	4		2	4.17	
S 9098	P	9	9			3.71	
S 9099	NP	0				----	
S 9100	P	14	14			3.44	
S 9101	P	14	13		1	3.88	
S 9102	P	7	7			4.13	
S 9103	P	11	11			3.93	
S 9104	P	10	8		2	3.32	
S 9105	P	8	8			3.43	
S 9106	P	12	12			4.02	
S 9107	P	10	10			4.12	
S 9108	NP	0				----	
S 9109	P	12	12			3.95	
S 9110	P	10	9		1	3.98	
S 9111	P	8	8			3.92	
S 9112	P	12	12			3.90	
S 9113	P	14	14			3.83	
S 9114	P	11	11			3.44	
S 9115	P	18	17		1	2.32	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 32 | Appendix II | Date March 31, 1972

Material Aspirin | Reproduction Data in Rats (Individual) | Laboratory No. 0729

Dose 250 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
A 9091	P	13	8		5	2.15	
A 9092	P	12			12	----	
A 9093	P	11		11		----	Died Day 7.
A 9094	P	13	1		12	1.79	
A 9095	P	11	2		9	1.76	
A 9096	P	14	14			3.12	
A 9097	P	14	14			3.07	
A 9098	P	12			12	----	
A 9099	NP	0				----	
A 9100	P	13	13			2.58	
A 9101	P	13			13	----	
A 9102	P	12	10		2	2.20	
A 9103	P	7	1		6	1.70	
A 9104	P	10	8		2	1.75	
A 9105	P	11		1	10	----	
A 9106	P	15	10		5	2.36	
A 9107	P	9	9			2.60	
A 9108	P	10	10			2.49	
A 9109	P	11	11			2.55	
A 9110	P	6			6	----	
A 9111	P	11	11			3.86	
A 9112	NP	0				----	Died Day 8.
A 9113	P	11	8		3	2.83	
A 9114	P	12		12		----	
A 9115	P	11	11			2.66	
A 9116	P	9	9			2.49	
A 9117	P	14			14	----	
A 9118	P	8	6		2	2.51	
A 9120	P	11			11	----	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 33 | Appendix II | Date March 31, 1972

Material FDA 71-3 | Reproduction Data in Rats (Individual) | Laboratory No. 0729 d

Dose 40 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
D 9001	P	10	9		1	3.68	
D 9002	P	13	13			3.88	
D 9003	P	13	13			4.10	
D 9004	P	9	9			3.79	
D 9005	P	11	10		1	3.82	
D 9006	P	2	2			4.12	
D 9007	P	10	10			3.85	
D 9008	P	13	13			3.52	
D 9009	P	12	12			3.74	
D 9010	P	15	15			3.46	
D 9011	P	8	8			3.69	
D 9012	P	12	12			4.10	
D 9013	P	14	13		1	3.98	
D 9014	P	7	6		1	4.01	
D 9015	P	8	7		1	4.20	
D 9016	NP	0				----	Died Day 10.
D 9017	P	9	9			4.06	
D 9018	P	7	7			5.49	
D 9019	P	12	11		1	3.92	
D 9020	P	13	13			4.11	
D 9021	P	10	10			3.84	
D 9022	P	11	10		1	3.42	
D 9023	P	11	11			3.52	
D 9024	P	10	10			5.09	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 34 | Appendix II | Date March 31, 1972

Material FDA 71-3 | Reproduction Data in Rats (Individual) | Laboratory No. 0729 d

Dose 100 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
D 9031	P	9	9			4.02	
D 9032	P	8	8			4.25	
D 9033	P	11	11			4.10	
D 9034	NP	0				----	Died Day 14
D 9035	P	11	11			3.82	
D 9036	P	12	11		1	3.46	
D 9037	P	11	11			3.60	
D 9038	P	11	11			5.24	
D 9039	P	13	11		2	3.95	
D 9040	P	10	10			3.25	
D 9041	P	13	13			3.07	
D 9042	P	13		13		----	Died Day 7.
D 9043	P	8	7		1	3.79	
D 9044	P	4	4			4.07	
D 9045	P	11	11			3.50	
D 9046	P	11	11			4.99	
D 9047	P	9	9			3.82	
D 9048	P	8	7		1	3.84	
D 9049	P	12	12			3.64	
D 9050	P	12	12			3.88	
D 9051	P	9	9			4.35	
D 9052	P	11	8		3	3.45	
D 9053	P	8	8			3.37	
D 9054	P	7	5		2	4.25	
D 9055	P	10	10			4.41	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 35 | Appendix II | Date March 31, 1972

Material FDA 71-3 | Reproduction Data in Rats (Individual) | Laboratory No. 0729 d

Dose 240 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
D 9061	P	12	12			3.96	
D 9062	P	9	9			4.30	
D 9063	P	14	14			3.24	
D 9064	P	9	9			4.23	
D 9065	P	9	8		1	3.79	
D 9066	P	14	12		2	3.39	
D 9067	P	15	10		5	3.47	
D 9068	NP	0				----	
D 9069	P	13	13			3.20	
D 9070	P	10	10			3.16	
D 9071	P	12	12			3.17	
D 9072	P	10	10			3.66	
D 9073	P	9	9			3.62	
D 9074	NP	0				----	
D 9075	P	10	10			3.62	
D 9076	P	7	7			5.70	
D 9077	P	10	10			3.80	
D 9078	P	8	7		1	3.89	
D 9079	P	13	12		1	3.50	
D 9080	P	13	12		1	3.21	
D 9081	P	10	10			4.48	
D 9082	NP	0				----	Died Day 9.
D 9083	P	14	13		1	3.46	
D 9084	NP	0				----	
D 9085	P	15			15	----	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 36 | Appendix II | Date March 31, 1972

Material FDA 71-3 | Reproduction Data in Rats (Individual) | Laboratory No. 0729 d

Dose 600 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
D 9091	NP	0				----	
D 9092	NP	0				----	
D 9093	P	13	13			2.80	
D 9094	P	13	3		11	1.64	
D 9095	NP	0				----	
D 9096	P	14	14			3.71	
D 9097	P	11	10		1	3.16	
D 9098	P	5	5			3.71	
D 9099	P	10	10			3.56	
D 9100	P	10	10			3.08	
D 9101	P	16	16			----	Died Day 7.
D 9102	P	10	10			3.64	
D 9103	P	13	13			3.98	
D 9104	P	11	11			3.35	
D 9105	P	9	9			3.65	
D 9106	P	10	10			3.03	
D 9107	P	9	9			4.00	
D 9108	P	11	11			4.05	
D 9109	P	10	10			3.54	
D 9110	P	11	9		2	3.57	
D 9111	P	12	9		3	3.26	
D 9112	P	15	15			2.75	
D 9113	P	13	13			2.49	
D 9114	P	9	4		5	1.69	
D 9115	P	9	8		1	2.57	
D 9116	NP	0				----	
D 9117	P	12	11		1	2.61	
D 9118	P	10	9		1	2.81	
D 9119	P	15	14		1	3.11	
D 9120	P	10	10			3.35	

* P = Pregnant; NP = Not Pregnant

Food and Drug Research Laboratories
INCORPORATED

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DRAFT REPORT

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date March 31, 1972

Laboratory No. 0730 d
Contract No. FDA 71-260

Sample: Fine light tan powdered material

Marking: FDA 71-3 (Sodium carragheenate)

Examination Requested: Teratologic evaluation of FDA 71- 3 in hamsters

Procedure: See Appendix I

Results: See Tables 1 through 4 and Appendix II

Conclusion: Subject to reexamination in the light of later findings, the following is concluded:

"The administration of the test material in graded dosage levels to pregnant hamsters for 5 consecutive days had no clearly significant effect on nidation or on maternal or fetal survival. There was a suggestion of delayed skeletal maturation (missing or incomplete centers of ossification). The number of malformations (terata?) seen were within the range of normal variation for the species. The skeletal findings are being reviewed prior to issuance of the final report on this compound.

(See following page for comment)

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Kenneth Morgareidge

Kenneth Morgareidge, Ph.D.
Vice President

Comment: Attention is called to the fact that this is the fourth of a series of reports which will be issued in accordance with the terms of the contract cited above. Eventually, a total of at least 36 compounds will have been tested in 18 pairs; each pair being run concurrently against one sham-treated control and one positive control group. Because of the inherent variability of biological data of the type dealt with here, the accumulation and pooling of sequential sets of control values will greatly enhance the statistical value of the findings and the ultimate reliability of the test results.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups: 31 through 36

Material: FDA 71-3

Table 1

Fate Summary
(Hamsters)

Date March 31, 1972

Laboratory No. 0730 d

Group	Material	Dose mg/kg	Total		At Term	
			Mated	Pregnant	Surviving (Total)	Number Pregnant
31	Sham	0	30	30	29	29
32	Aspirin*	250	30	27	29	26
33	FDA 71-3	10	26	26	26	26
34	FDA 71-3	45	26	24	26	24
35	FDA 71-3	470	26	24	26	24
36	FDA 71-3	900	30	27	27	26

* Positive Control

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group: 31 through 36

Material: FDA 71-3

Date March 31, 1972

Laboratory No. 0730 d

Table 2
Reproduction Data
(Hamsters)

Group:	31	32	33	34	35	36
Dose (mg/kg):	Sham	Aspirin**	10	45	470	900
Pregnancies						
Total No.	30	27	26	24	24	27
Died or Aborted (before Day 14)	1	1	0	0	0	2
To term (on Day 14)	29	26	26	24	24	26
Live litters						
Total No. *	29	24	26	22	24	26
Implant sites						
Total No.	340	327	319	293	288	323
Average/dam *	11.7	12.6	12.3	12.2	12.0	12.4
Resorptions						
Total No. *	15	31	9	34	16	6
Dams with 1 or more sites resorbed	8	12	8	11	10	6
Dams with all sites resorbed	0	2	0	2	0	0
Per cent partial resorptions	27.6	46.2	30.8	45.8	41.7	25.0
Per cent complete resorptions	-	7.69	0	8.33	-	-
Live fetuses						
Total No.	322	296	310	251	271	317
Average/dam *	11.1	11.4	11.9	10.5	11.3	12.2
Dead fetuses						
Total No. *	3	0	0	8	1	0
Dams with 1 or more dead	3	-	-	3	1	-
Dams with all dead	0	-	-	0	0	-
Per cent partial dead	10.3	-	-	12.5	4.17	-
Per cent all dead	-	-	-	-	-	-
Average fetus weight, g	1.86	1.78	1.82	1.81	1.75	1.78

* Includes only those dams examined at term.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 31 through 36 — Table 3 — Laboratory No. 0730 d

Material FDA 71-3 — Date March 31, 1972

Summary of Skeletal Findings*

(Hamsters)

Findings	Group No. / Dose (mg/kg)	31 Sham	32 Aspirin**	33 10	34 45	35 470	36 900
Live Fetuses Examined		213/28	206/24	216/26	174/21	190/24	223/
Sternebrae							
Incomplete oss.		Data will appear in final report					
Scrambled							
Bipartite		30/19	25/19	46/22	30/14	25/13	31/
Fused							
Extra							
Missing		53/20	81/20	75/22	64/18	89/22	135/
Other							
Ribs							
Incomplete oss.							
Fused/split		2/2			3/1	3/3	2/
Wavy			1/1				1/
Less than 12							
More than 13		19/10	11/6	27/17	21/10	26/10	30/
Other							
Vertebrae							
Incomplete oss.		2/2	2/2		4/2	3/2	5/
Scrambled							
Fused							
Extra ctrs. oss.		23/14	12/8	6/6	9/4	10/7	7/
Scoliosis		4/4	4/4	1/1	5/4	4/4	11/
Tail defects							
Other							
Skull		1/1					
Incomplete closure							
Missing							
Craniostosis			1/1				
Other, Occip./Parietals/ Facials;inc.		3/3	2/2		2/1	2/2	4/
Extremities							
Incomplete oss.		36/14	55/17	45/14	38/12	72/19	65/
Missing							
Extra							
Miscellaneous							
Pubis/Ilium/Ischium;inc.		3/3	2/2				6/
Hind, left leg rotation			1/1				1/
Hyoid; missing			1/1		1/1		2/
Exencephaly						2/2	2/
Cleft Palate							1/

* Numerator=Number of fetuses affected; Denominator=Number of litters affect
** Positive control: 250 mg/kg.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 31 through 36

Species Hamsters

Table 4

Average Body Weights *

Date March 31, 1972

Laboratory No. 0730 d

Group	Material	Dose Level	Day 0	6	8	10	14	
		mg/kg	g					
31	Sham	0	97.4	103	104	116	137	(29)
32	Aspirin	250	99.7	104	105	117	135	(26)
33	FDA 71-3	10	101	105	110	120	143	(26)
34	FDA 71-3	45	102	106	109	118	140	(24)
35	FDA 71-3	470	99.2	104	104	114	137	(24)
36	FDA 71-3	900	98.0	103	105	116	142	(26)

* Of pregnant dams

** Number of surviving dams in parentheses (c.f. Table 1)

Appendix I

Teratology Study in Hamsters

Virgin adult female golden hamsters from an outbred strain were individually housed in mesh bottom cages in temperature and humidity controlled quarters with free access to food and fresh tap water at all times. They were mated (1 to 1) with mature males and the appearance of motile sperm in the vaginal smear was considered as Day 0 of gestation. Beginning on Day 6 and continuing daily through Day 10 of gestation, the indicated dose levels of the test material were administered by oral intubation; the controls were sham-treated.

Body weights were recorded on Days 0, 8, 10, and 14 of the gestation period. All animals were observed daily for appearance and behavior with particular attention to food consumption in order to better recognize any abnormalities resulting from anorexic effects in the pregnant animal.

On Day 14, all animals were subjected to Caesarian section under deep anesthesia and the numbers of implantation sites, resorption sites, live and dead fetuses were recorded. All live pups were weighed and the genital tract of each dam was examined for any anatomical abnormalities.

All fetuses were examined grossly for the presence of external congenital defects and one-third of each litter underwent detailed visceral examination under 10X magnification. The remaining two-thirds of the pups were cleared in potassium hydroxide, stained with alizarin red dye, and examined for the presence of sketal abnormalities.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 31 | Appendix II | Date March 31, 1972

Material Sham | Reproduction Data in Hamsters (Individual) | Laboratory No. 0730

Dose 0

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
S 0091	P	12	11	1	0	2.04	
S 0092	P	11	11	0	0	1.75	
S 0093	P	12	12	0	0	2.03	
S 0094	P	9	9	0	0	1.85	
S 0095	P	11	11	0	0	1.81	
S 0096	P	14	12	0	2	1.72	
S 0097	P	12	12	0	0	1.94	
S 0098	P	11	7	0	4	1.58	
S 0099	P	11	11	0	0	1.71	
S 0100	P	14	14	0	0	1.69	
S 0101	P	11	10	0	1	1.77	
S 0102	P	13	13	0	0	1.90	
S 0103	P	13	13	0	0	1.85	
S 0104	P	10	10	0	0	1.79	
S 0105	P	12	12	0	0	1.97	
S 0106	P	13	11	0	2	1.94	
S 0107	P	14	13	1	0	1.74	
S 0108	P	10	9	0	1	1.63	
S 0109	P	12	0	12	0	--	Died Day 14.
S 0110	P	12	9	0	3	1.91	
S 0111	P	11	9	1	1	2.16	
S 0112	P	11	11	0	0	1.64	
S 0113	P	11	11	0	0	1.84	
S 0114	P	12	12	0	0	1.64	
S 0115	P	8	8	0	0	2.20	
S 0116	P	12	11	0	1	1.82	
S 0117	P	14	14	0	0	1.82	
S 0118	P	13	13	0	0	2.11	
S 0119	P	11	11	0	0	2.03	
S 0120	P	12	12	0	0	2.11	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 32 | Appendix II | Date March 31, 1972

Material Aspirin | Reproduction Data in Hamsters (Individual) | Laboratory No. 0730

Dose 250 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
A 0091	P	9	9	0	0	1.78	
A 0092	P	14	14	0	0	1.88	
A 0093	P	11	11	0	0	1.76	
A 0094	P	12	10	0	2	2.08	
A 0095	NP	0				--	
A 0096	P	15	14	0	1	1.81	
A 0097	P	13	13	0	0	1.83	
A 0098	NP	0				--	
A 0099	P	13	11	0	2	1.82	
A 0100	P	10	10	0	0	1.90	
A 0101	P	12	12	0	0	1.56	
A 0102	P	15	14	0	1	1.83	
A 0103	P	16	15	0	1	1.50	
A 0104	P	9	8	0	1	1.97	
A 0105	P	11	11	0	0	1.96	
A 0106	P	19	18	0	1	1.57	
A 0107	P	13	11	0	2	1.82	
A 0108	P	12	12	0	0	1.60	
A 0109	P	9	0	0	9	--	
A 0110	P	14	14	0	0	1.65	
A 0111	P	13	13	0	0	1.70	
A 0112	P	15	0	15	0	--	Dam Died Day 8.
A 0113	NP	0				--	
A 0114	P	12	12	0	0	1.65	
A 0115	P	12	11	0	1	1.91	
A 0116	P	9	0	0	9	--	
A 0117	P	15	15	0	0	1.72	
A 0118	P	15	14	0	1	1.69	
A 0119	P	11	11	0	0	1.91	
A 0120	P	13	13	0	0	1.80	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 33 | Appendix II | Date March 31, 1972

Material FDA 71-3 | Reproduction Data in Hamsters (Individual) | Laboratory No. 0730 d

Dose 10 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
D 0001	P	11	11	0	0	1.90	
D 0002	P	11	10	0	1	1.79	
D 0003	P	11	10	0	1	1.69	
D 0004	P	13	13	0	0	1.94	
D 0005	P	14	14	0	0	1.73	
D 0006	P	13	13	0	0	1.81	
D 0007	P	14	14	0	0	1.83	
D 0008	P	14	13	0	1	1.69	
D 0009	P	12	12	0	0	1.78	
D 0010	P	11	11	0	0	1.72	
D 0011	P	16	16	0	0	1.97	
D 0012	P	11	11	0	0	1.96	
D 0013	P	10	10	0	0	1.86	
D 0014	P	13	13	0	0	2.08	
D 0015	P	13	13	0	0	1.80	
D 0016	P	12	12	0	0	1.77	
D 0017	P	12	11	0	1	1.73	
D 0018	P	13	13	0	0	1.78	
D 0019	P	11	9	0	2	1.93	
D 0020	P	11	11	0	0	1.92	
D 0021	P	8	8	0	0	1.68	
D 0022	P	14	13	0	1	1.71	
D 0023	P	12	12	0	0	1.65	
D 0024	P	16	15	0	1	1.75	
D 0025	P	12	11	0	1	1.90	
D 0026	P	11	11	0	0	1.92	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 34 — Appendix II — Date March 31, 1972

Material FDA 71-3 — Reproduction Data in Hamsters (Individual) — Laboratory No. 0730 d

Dose 45 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
D 0031	P	13	13	0	0	1.90	
D 0032	P	12	12	0	0	1.98	
D 0033	P	12	11	0	1	1.54	
D 0034	P	17	17	0	0	1.79	
D 0035	P	13	12	0	1	1.78	
D 0036	P	9	0	0	9	--	
D 0037	P	11	9	1	1	1.72	
D 0038	P	11	11	0	0	1.99	
D 0039	P	11	7	1	3	1.45	
D 0040	P	14	12	0	2	2.02	
D 0041	P	10	10	0	0	1.53	
D 0042	P	13	13	0	0	1.89	
D 0043	P	15	15	0	0	1.98	
D 0044	P	11	11	0	0	1.56	
D 0045	NP	0				--	
D 0046	P	12	12	0	0	2.03	
D 0047	P	7	0	0	7	--	
D 0048	P	11	10	0	1	1.96	
D 0049	P	11	9	0	2	1.90	
D 0050	P	14	14	0	0	1.84	
D 0051	P	15	15	0	0	1.80	
D 0052	P	14	13	0	1	1.86	
D 0053	P	13	13	0	0	2.08	
D 0054	P	11	11	0	0	2.19	
D 0055	NP	0				--	
D 0056	P	13	1	6	6	1.12	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 35 — Appendix II — Date March 31, 1972

Material FDA 71-3 — Reproduction Data in Hamsters (Individual) — Laboratory No. 0730 d

Dose 470 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
D 0061	P	9	9	0	0	1.78	
D 0062	P	9	9	0	0	2.00	
D 0063	P	12	12	0	0	1.70	
D 0064	P	13	13	0	0	1.85	
D 0065	P	14	13	0	1	1.82	
D 0066	P	11	11	0	0	1.76	
D 0067	NP	0				--	
D 0068	P	13	10	0	3	1.66	
D 0069	P	12	10	0	2	1.74	
D 0070	P	15	15	0	0	1.62	
D 0071	P	15	14	0	1	1.79	
D 0072	P	15	13	1	1	1.81	
D 0073	P	6	6	0	0	1.84	
D 0074	P	10	8	0	2	1.57	
D 0075	P	13	13	0	0	1.91	
D 0076	P	13	13	0	0	1.65	
D 0077	P	13	13	0	0	1.94	
D 0078	P	13	13	0	0	1.76	
D 0079	P	11	10	0	1	1.63	
D 0080	P	10	10	0	0	1.62	
D 0081	P	16	13	0	3	1.80	
D 0082	NP	0				--	
D 0083	P	9	9	0	0	1.65	
D 0084	P	12	11	0	1	1.73	
D 0085	P	11	10	0	1	1.63	
D 0086	P	13	13	0	0	1.72	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 36 | Appendix II | Date March 31, 1972

Material FDA 71-3 | Reproduction Data in Hamsters (Individual) | Laboratory No. 0730 d

Dose 900 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
			Alive	Dead			
D 0091	P	15	15	0	0	1.95	
D 0092	P	14	14	0	0	1.79	
D 0093	P	11	11	0	0	1.70	
D 0094	P	13	13	0	0	1.98	
D 0095	P	12	11	0	1	1.68	
D 0096	P	11	11	0	0	1.89	
D 0097	NP	0				--	
D 0098	P	13	13	0	0	1.33	
D 0099	P	11	10	0	1	1.85	
D 0100	P	14	14	0	0	**	
D 0101	P	13	13	0	0	**	
D 0102	P	9	9	0	0	2.19	
D 0103	P	10	10	0	0	1.87	
D 0104	P	9	9	0	0	1.68	
D 0105	P	11	11	0	0	1.92	
D 0106	P	12	12	0	0	1.75	
D 0107	P	11	11	0	0	1.97	
D 0108	P	13	12	0	1	1.94	
D 0109	P	13	12	0	1	1.46	
D 0110	P	12	11	0	1	1.78	
D 0111	P	14	0	14	0	--	Dam died Day 7
D 0112	P	15	15	0	0	1.55	
D 0113	NP	0				--	Dam died Day 7
D 0114	NP	0				--	
D 0115	P	14	14	0	0	1.74	
D 0116	P	13	13	0	0	1.79	
D 0117	P	14	14	0	0	1.72	
D 0118	P	13	13	0	0	1.85	
D 0119	P	14	13	0	1	1.66	
D 0120	P	13	13	0	0	1.69	

* P = Pregnant; NP = Not Pregnant

** Not weighed

Food and Drug Research Laboratories
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DRAFT REPORT

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date March 31, 1972

Laboratory No. 0731 d
Contract No. FDA 71-260

Sample: Fine light tan powdered material

Marking: FDA 71-3 (Sodium carragheenate)

Examination Requested: Teratologic evaluation of FDA 71- 3 in rabbits

Procedure: (See Appendix I)

Results: See Tables 1 through 4 and Appendix II

Conclusion: Attention is called to the fact that this is the fourth of a series of reports which will be issued in accordance with the terms of the contract cited above. Eventually, a total of at least 36 compounds will have been tested in 18 pairs; each pair being run concurrently against one sham-treated control and one positive control group. Because of the inherent variability of biological data of the type dealt with here, the accumulation and pooling of sequential sets of control values will greatly enhance the statistical value of the findings and the ultimate reliability of the test results.

For these reasons, the conclusion stated below is regarded as provisional and subject to reexamination in the light of later findings:

"The administration of up to 600 mg/kg (body weight) of the test material to pregnant rabbits for 13 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Kenneth Morgareidge, Ph.D.
Vice President

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups: 31 through 36

Material: FDA 71-3

Table 1

Fate Summary
(Rabbits)

Date March 31, 1972

Laboratory No. 0731 d

Group	Material	Dose mg/kg	Total		At Term	
			Mated	Pregnant	Surviving (Total)	Number Pregnant
31	Sham	0	15	13	11	9
32	6-AN*	2.5	15	13	15	13
33	FDA 71-3	40	15	13	12	9
34	FDA 71-3	100	15	13	15	13
35	FDA 71-3	240	15	13	13	11
36	FDA 71-3	600	15	12	12	9

* Positive control: 6- amino nicotinamide dosed on Day 9.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group: 31 through 36 Date March 31, 1972

Material: FDA 71-3 Laboratory No. 0731 d

Table 2
Reproduction Data
(Rabbits)

Group:	31	32	33	34	35	36
Dose (mg/kg):	Sham	6-AN**	40	100	240	600
Pregnancies						
Total No.	13	13	13	13	13	12
Died or aborted (before Day 29)	4	0	3	0	2	3
To term (on Day 29)	9	13	9	13	11	9
Corpora lutea						
Total No.	243	235	273	304	249	216
Average/dam mated	16.2	15.7	18.2	20.3	16.6	14.4
Live litters						
Total No.*	9	10	8	12	9	7
Implant sites						
Total No. (at term)	39	59	52	68	63	50
Average/dam*	4.33	4.54	5.78	5.23	5.73	5.56
Resorptions						
Total No.*	5	15	15	5	9	15
Dams with 1 or more sites resorbed	3	8	4	3	6	4
Dams with all sites resorbed	0	2	2	1	2	2
Per cent partial resorptions	33.3	61.5	44.4	23.1	54.5	44.4
Per cent complete resorptions	--	15.4	22.2	7.69	18.2	22.2
Live fetuses						
Total No. (at term)	34	43	37	62	54	35
Average/dam*	3.78	3.31	4.11	4.77	4.91	3.89
Dead fetuses						
Total No.*	0	1	0	1	0	0
Dams with 1 or more dead	-	1	-	1	-	-
Dams with all dead	-	0	-	0	-	-
Per cent partial dead	-	7.69	-	7.69	-	-
per cent all dead	-		-	-	-	-
Average fetus weight, g	37.3	31.0	42.3	39.9	35.8	38.6

* Includes only those dams examined at term.

** Positive control: 2.5 mg/kg 6-amino nicotinamide dosed on Day 9.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 31 through 36 | Table 3 | Laboratory No. 0731 d

Material FDA 71-3 | Date March 31, 1972

Summary of Skeletal Findings*
(Rabbits)

Findings	Group No.	31	32	33	34	35	36
	Dose (mg/kg)	Sham	6-AN**	40	100	240	600
Live Fetuses Examined		32/9	42/10	37/8	62/12	54/9	35/7
Sternebrae							
Incomplete oss.		7/5	16/7	10/3	9/5	13/7	5/3
Scrambled							
Bipartite			8/6	1/1			1/1
Fused		2/1	12/6			3/3	
Extra			3/3	2/1		6/4	4/3
Missing			1/1				
Other							
Ribs							
Incomplete oss.			2/2				
Fused/split			7/6				
Wavy							
Less than 12							
More than 13		1/1	1/1	6/2	1/1		1/1
Other							
Vertebrae							
Incomplete oss.							
Scrambled			1/1				
Fused							
Extra ctrs. oss.							
Scoliosis			1/1				
Tail defects			23/7			3/1	
Other							
Skull							
Incomplete closure							
Missing							
Craniostosis		1/1	8/2	1/1	9/4	5/3	5/3
Other; Eyes; incomplete			8/2				
Extremities							
Incomplete oss.							
Missing							
Extra							
Miscellaneous							
Club feet			5/3				

* Numerator=Number of fetuses affected; Denominator=Number of litters affecte
** Positive control: 6- amino nicotinamide dosed on Day 9

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 31 through 36 Date March 31, 1972

Material FDA 71-3 Laboratory No. 0731 d

Table 3-a

Summary of Soft Tissue Abnormalities

(Rabbits)

Group	Material	Dose level mg/kg	Dam	Number of Pups	Description
31	Sham	0	S-1051	2	Euryopia
				1	Hair lip
32	6-AN *	2.5	Z-1046	5	Anopia
				3	Club feet
32	6-AN		Z-1047	7	Anopia
				3	Cleft palate
				2	Hair lip
32	6-AN		Z-1049	6	Anopia
				4	Club feet
				3	Cleft palate
				3	Hair lip
32	6-AN		Z-1052	1	Anopia
				2	Missing digits
				1	Dysgnathia
32	6-AN		Z-1053	2	Anopia
				3	Club feet
32	6-AN		Z-1054	3	Club feet
				1	Anopia
				1	Hair lip
32	6-AN		Z-1055	7	Anopia
				5	Club feet
32	6-AN		Z-1059	5	Anopia
				3	Club feet
				3	Cleft palate

* 6- amino nicotinamide dosed on Day 9.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 31 through 36

Species Rabbits

Table 4

Average Body Weights

Date March 31, 1972

Laboratory No. 0731 d

Group	Material	Dose Level	Day 0	6	12	18	29 **
		mg/kg	kg				
31	Sham	0	2.34	2.35	2.38	2.36	2.41 (9)
32	6-AN***	2.5	2.21	2.18	2.27	2.27	2.36 (13)
33	FDA 71-3	40	2.25	2.29	2.34	2.27	2.48 (9)
34	FDA 71-3	100	2.30	2.33	2.37	2.41	2.48 (13)
35	FDA 71-3	240	2.48	2.52	2.51	2.51	2.47 (11)
36	FDA 71-3	600	2.50	2.60	2.61	2.69	2.60 (9)

* Of pregnant dams
** Number of surviving dams in parentheses (c.f. Table 1)
*** Positive control: 6 amino-nicotinamide dosed on Day 9.

Appendix I

Teratology Study in Rabbits

Virgin, adult, Dutch-belted female rabbits were individually housed in mesh bottom cages in temperature and humidity-controlled quarters with free access to food and fresh tap water. On Day 0, each doe was given an injection of 0.4 ml of human chorionic gonadotropin (400 IU) via the marginal ear vein. Three hours later, each doe was inseminated artificially with 0.3 ml of diluted semen from a proven donor buck using approximately 20×10^6 motile sperm according to the procedure described by Vogin et al (Pharmacologist 11, 282 (1969)). Beginning on Day 6 and continuing daily through Day 18 the females were dosed with the indicated dosages by oral intubation; the controls were sham treated.

Body weights were recorded on Days 0, 6, 12, 18, and 29 of gestation. All animals were observed daily for appearance and behavior, with particular attention to food consumption and body weight in order to rule out any abnormalities which may have occurred as a result of anorexic effects in the pregnant female animal.

On Day 29 all does were subjected to Caesarean section under surgical anesthesia, and the numbers of corpora lutea, implantation sites, resorption sites and live and dead fetuses were recorded. Body weights of the live pups were also recorded. The urogenital tract of each animal was examined in detail for normality. In addition all fetuses underwent a detailed gross examination for the presence of external congenital abnormalities. The live fetuses of

each litter were then placed in an incubator for 24 hours for the evaluation of neonatal survival. All surviving pups were sacrificed, and all pups examined for visceral abnormalities (by dissection). All fetuses were then cleared in potassium hydroxide (KOH), stained with alizarin red S dye and examined for skeletal defects.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 31 | Appendix II | Date March 31, 1972

Material Sham | Reproduction Data in Rabbits (Individual) | Laboratory No. 0731

Dose 0

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead			
S 1046	P	13	4		4		----	Died Day 14
S 1047	P	19	1	1			36.4	
S 1048	NP	12	0				----	
S 1049	P	19	5	5			36.9	
S 1050	P	25	5	3		2	43.6	
S 1051	P	15	6	5		1	17.7	
S 1052	P	8	6	6			42.7	
S 1053	P	16	6	6			32.8	
S 1054	P	20	3	3			38.4	
S 1055	P	19	1	1			46.1	
S 1056	NP	2	0				----	
S 1057	P	13	4		4		----	Died Day 10
S 1058	P	23	6	4		2	40.7	
S 1059	P	20	5		5		----	Died Day 10
S 1060	P	19	2		2		----	Died Day 11

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 32 | Appendix II | Date March 31, 1972

Material 6 - AN | Reproduction Data in Rabbits (Individual) | Laboratory No. 0731

Dose 2.5 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead			
Z 1046	P	21	6	6			28.8	
Z 1047	P	18	7	7			27.0	
Z 1048	NP	8	0				----	
Z 1049	P	21	6	5		1	29.2	
Z 1050	P	11	1			1	----	
Z 1051	P	8	2			2	----	
Z 1052	P	9	4	1		3	21.6	
Z 1053	P	11	4	3		1	29.7	
Z 1054	P	39	6	3		3	29.0	
Z 1055	P	25	8	7	1		27.6	
Z 1056	P	15	5	4		1	34.0	
Z 1057	P	8	2	2			43.0	
Z 1058	P	14	3			3	----	
Z 1059	P	23	5	5			40.3	
Z 1060	NP	4	0				----	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 33 | Appendix II | Date March 31, 1972

Material FDA 71-3 | Reproduction Data in Rabbits (Individual) | Laboratory No. 0731 d

Dose 40 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead			
D 1001	P	31	7	7			37.2	
D 1002	P	19	6	6			37.3	
D 1003	P	14	5			5	----	
D 1004	NP	10	0				----	
D 1005	P	26	4	4			40.3	
D 1006	NP	5	0				----	
D 1007	P	7	3	3			43.4	
D 1008	P	10	2	1		1	56.9	
D 1009	P	24	7	6		1	37.3	
D 1010	NP	5	0				----	
D 1011	P	10	3	3			42.3	
D 1012	P	21	5		5		----	Died Day 24
D 1013	P	20	5		5		----	Died Day 21
D 1014	P	44	7	7			43.3	
D 1015	P	27	8			8	----	Died Day 25

P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 34 | Appendix II | Date March 31, 1972

Material FDA 71-3 | Reproduction Data in Rabbits (Individual) | Laboratory No. 0731 d

Dose 100 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead			
D 1016	P	15	2	2			44.3	
D 1017	P	16	7	7			40.6	
D 1018	P	20	3	3			46.1	
D 1019	P	22	6	6			41.3	
D 1020	P	31	10	9	1		36.5	
D 1021	P	16	3	3			42.9	
D 1022	P	18	8	8			30.6	
D 1023	NP	7	0				----	
D 1024	P	5	3			3	----	
D 1025	P	26	5	4		1	41.1	
D 1026	P	22	5	5			38.5	
D 1027	NP	3					----	
D 1028	P	21	8	8			36.2	
D 1029	P	14	7	6		1	41.3	
D 1030	P	16	1	1			**	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 35

Appendix II

Date March 31, 1972

Material FDA 71-3

Reproduction Data in Rabbits (Individual)

Laboratory No. 0731 d

Dose 240 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead			
D 1031	P	13	8	7		1	30.0	
D 1032	P	23	7	7			39.2	
D 1033	P	21	3	3			42.9	
D 1034	P	16	6	5		1	32.9	
D 1035	NP	16	0				----	
D 1036	P	34	10	9		1	36.4	
D 1037	P	28	6	6			36.4	
D 1038	P	20	7	7			30.4	
D 1039	P	22	5	5			36.6	
D 1040	NP	3					----	
D 1041	P	5	4			4	----	
D 1042	P	7	1			1	----	
D 1043	P	14	6	5		1	37.8	
D 1044	P	15	3		3		----	Died Day 13
D 1045	P	12	7		7		----	Died Day 29

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 36 | Appendix II | Date March 31, 1972

Material FDA 71-3 | Reproduction Data in Rabbits (Individual) | Laboratory No. 0731 d

Dose 600 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses		Resorption Sites	Average Fetus Weight (g)	Remarks
				Alive	Dead			
D 1046	P	15	7			7	----	
D 1047	NP	9	0				----	
D 1048	P	23	6	5		1	35.4	
D 1049	P	16	3			3	----	
D 1050	P	17	2	2			36.0	
D 1051	NP	4	0				----	
D 1052	P	15	6				----	Died Day 14
D 1053	P	9	1	1			50.2	
D 1054	NP	14	0				----	
D 1055	P	19	8	8			31.3	
D 1056	P	11	4	4			46.4	
D 1057	P	20	8		8		----	Died Day 10
D 1058	P	20	10	10			32.0	
D 1059	P	8	3		3		----	Died Day 12
D 1060	P	16	9	5		4	39.0	

* P = Pregnant; NP = Not Pregnant

Company Code: [redacted]
Sub. No. 0067

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Institute of Experimental Pathology and Toxicology

Albany Medical College

Albany, New York 12208

CONFIDENTIAL

SAFETY EVALUATION OF CARRAGEENAN

Final Report

July 1971

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SUMMARY

Two forms of carrageenan, namely a native form derived from *Chondrus crispus* (HMR) and a degraded form from *Eucheuma spinosum* (C16) were administered, principally in the drinking water, to guinea pigs, rats, gerbils and rhesus monkeys (*Macaca mulatta*) for various lengths of time. Recovery experiments were also carried out in some instances.

The presence of 5% or 2% C16 in the drinking water of guinea pigs (corresponding to daily doses of approximately 6-8 and 3.3 g/kg C16 respectively for 35 days) elicited ulceration of the cecum and colon, with histopathologic changes corresponding closely to published descriptions of these lesions but lacking crypt abscesses and epithelial hyperplasia.

In rats, drinking water containing 5% C16 (corresponding to daily doses of approximately 6-10 g/kg) produced no detectable damage to the gastrointestinal tract over a period of 7 months despite the consistent presence of occult blood in the stools during this period. Gerbils consuming 5% C16 in the diet for 6 months (3.5 g/kg/day) appeared to be totally unaffected by the carrageenan.

Rhesus monkeys were given 2%, 1% or 0.5% C16 in the drinking water for periods of 7-11, 14 and 14 weeks respectively. The corresponding daily intakes of C16 were 2.9, 1.4, and 0.7 g/kg respectively. Changes ranging from ulceration of the cecum and colon at the high dose to erosion of epithelium at the two lower doses present a consistent dose-response relationship, for the limited number of animals involved (2 at each dose level).

The undegraded carrageenan (HMR) was given to guinea pigs and rats at a level of 1% in drinking water (approximately 1 g/kg/day and 1.3-1.8 g/kg/day respectively). Gerbils consumed 5% HMR in the diet (4.2 g/kg/day). In all instances, over periods up to 6 months, no effect was observed on the integrity of the gastrointestinal

epithelium, a finding reflected in the negative tests for fecal occult blood.

Six rhesus monkeys received 1% HMR in drinking water (corresponding to 1.3 g/kg/day) over periods of 7-11 weeks. Of two monkeys sacrificed at 11 weeks, only one had minimal changes in the colon whose relation to carrageenan ingestion is questionable. The other 4 monkeys were followed through a recovery period of 11 weeks, during which time all observations corresponded to those in controls. Subsequent administration to the same 4 monkeys of HMR by stomach tube in doses increasing to 1250 mg/kg/day for a total of 84 days left the animals clinically unaffected and at autopsy no gross nor histopathologic change was visible in gastrointestinal tract of any one of these monkeys.

INTRODUCTION

Nature and uses of carrageenan

Native carrageenan, used as a food additive, is extracted from the so-called "red" seaweeds and in general is made up of a gelling component called kappa-carrageenan and a non-gelling component called lambda-carrageenan (Furia, 1968). The ratio of the two fractions varies from one species to another, from one geographic location to another, and from one time of the year to another (Guiseley, 1968). A third component designated the iota-fraction has been extracted from certain carrageenan-bearing seaplants such as *Eucheuma spinosum* (Guiseley, 1968). The iota-fraction forms an elastic gel with calcium salts.

Since carrageenan is a sulphated polygalactose of high electronegativity, it reacts with positively charged polymers such as proteins, forming complexes, gels or precipitates (Guiseley, 1968). This property makes it extremely valuable to the food industry where it is used in the suspension of particles in chocolate milk, in the production of milk pudding, and in water-dessert gels. Carrageenan is an important ingredient of prepared infant formulae. Carrageenan is also used to thicken soups, sauces and gravies, and as a binder in dentifrices (Furia, 1968).

Carrageenan has found use as a therapeutic agent. In France, "Coreine", a native carrageenan, has been used in the treatment of colitis since 1911. "Ebimar", a product based on degraded carrageenan, has been used since 1960 for the treatment of peptic ulcer (Shirlaw, unpublished). For this purpose *Eucheuma spinosum* has been used exclusively.

Pharmacological actions of degraded carrageenans

Experimentally-produced gastroduodenal ulceration can be prevented by degraded carrageenans given orally (Anderson and Watt, 1959; Anderson and Soman, 1967a), intraduodenally (Anderson and Soman, 1963), or parenterally (Anderson and Soman, 1967b; Watt et al., 1966). The mechanism by which carrageenan protects against ulceration is not yet fully understood, although it has been related to the effect of the polysaccharide on gastric secretion. The mechanism of inhibition of peptic activity by carrageenan has been shown to be substrate depletion following interaction of inhibitor and substrate (Anderson, 1961; Anderson and Baillee, 1967). The inhibitory activity of carrageenan is dependent on its sulphur content and molecular size. Although undegraded carrageenan, (molecular weight of 800,000-1,000,000), inhibits pepsin activity more strongly than degraded carrageenan, molecular weight of 20,000-30,000), the activity of degraded carrageenan appears to be more stable under varying conditions such as different pH values (Anderson and Baillee, 1967). In addition, the degraded form disperses in water more readily than the undegraded form, rendering the low-molecular material more suitable as a therapeutic agent (Anderson and Baillee, 1967).

Studies of parenterally-administered carrageenan

The toxicity of carrageenan varies with the route of administration. Undegraded carrageenan, given intravenously to rabbits (50 mg) resulted in death within forty-eight hours. Diffuse renal cortical necrosis occurred and widespread capillary thrombosis developed in various organs (Morard, *et al.* 1964). Undegraded carrageenan given intravenously in a dose of 1 mg/kg to guinea pigs proved lethal in 30 minutes (Anderson and Soman, 1966).

Undegraded carrageenan (from *Chondrus crispus*) was found to induce the proliferation of fibroblasts, with subsequent formation of a connective tissue granuloma, when injected subcutaneously in guinea-pigs (Robertson and Schwartz, 1953). Experiments by Williams (1957) and Benitz and Hall (1959) demonstrated that similar changes could be elicited in the rat. Cater (1961) showed that a single injection of 5 ml of a 1% carrageenan solution given subcutaneously in young female rats resulted in loss of hair at the site of injection and fibrous degeneration of mammary gland epithelium with hyaline thickening of the capillary walls.

Recent investigations of carrageenan

Watt and Marcus (1969) reported that ten guinea pigs given 5% aqueous solution of degraded carrageenan (*E. spinosum*) as drinking water for twenty to thirty days all developed ulcerative lesions in the cecum, with some extension into the colon and rectum. Of ten guinea pigs given 1% aqueous solution of native carrageenan for the same length of time, eight developed cecal lesions. In later experiments, the same workers (Marcus and Watt, 1969) found that rabbits, mice and rats also develop ulcerative lesions of the cecum when given degraded carrageenan (obtained from *C. crispus* and *E. spinosum*). In mice, the changes were minimal in comparison with the other species studied.

Experiments by Sharratt *et al.* (1970, 1971) failed to confirm the development of lesions in rats, mice, ferrets and squirrel monkeys, although multiple cecal ulcerations were produced in guinea pigs by administration of 5% native carrageenan or 1% degraded carrageenan in the drinking water for two to four weeks. Other investigations have also failed to produce cecal ulceration in rats and mice by the use of degraded and native carrageenan (Dubrasquet, Lehy, and Bonfils, unpublished; Maillet, unpublished).

Relationships of ulceration in guinea pigs to other species

The issue of the relationship of carrageenan-induced cecal ulceration in the guinea pig to clinical ulcerative colitis occurring in man has proved to be controversial. Sharratt *et al.* (1970) state that the "macrophage response limitation of the lesion to the cecum, and absence of crypt abscesses and epithelial hyperplasia are features [which distinguish the guinea pig] from the lesion of ulcerative colitis in man." On the other hand Watt and Marcus (1970) and Mottet (1970) suggest that the presence of crypt abscesses and necrosis of the crypt epithelium involving all regions of the crypt are typical of the human disease.

In a comparative study of the cecum of the guinea pig, rat, mouse and man, Maillet (unpublished) found that the cecal mucosa of the guinea pig is readily distinguishable from that of other species because it contains macrophages, whereas the cecal mucosa of man, rat and mouse essentially contains only lymphocytes and plasmocytes. Maillet stresses the role of macrophages in the development of lesions in the guinea pig (see also Sharratt *et al.* (1970)) and feels that a similar lesion is unlikely to occur in man since the ceca of man and other species are functionally and morphologically different from that of the guinea pig.

Experience with carrageenan in man

It is estimated that about 50,000 patients in France have received degraded carrageenan at dosage levels up to 5 g/day as treatment for peptic ulceration (Bonfils, 1970). No untoward effect suggestive of ulcerative colitis has been encountered. Bonfils (1970) has reported that there has been no evidence of gastrointestinal disease developing in his 200 patients who are being given Ebimar® as treatment for peptic ulcer, and that no sign of ulceration of the large bowel appeared when degraded carrageenan was fed in very high doses to gastric ulcer patients. Marcus and Watt (1970b),

claim that because Ebimar contains aluminum and because results of tests for occult blood in the feces were not reported, there is insufficient evidence to claim that orally-administered carrageenan does not produce harmful effects in man.

FAO/WHO toxicological evaluation of carrageenan

The Joint FAO/WHO Expert Committee on Food Additives (1970) assigned to carrageenan and the related polysaccharide of marine origin, furcellaran, a combined acceptable daily intake of 0-500 mg/kg body weight. The basis of this toxicological evaluation is recorded in the monograph entitled "Carrageenan and Furcellaran" (FAO Nutrition Meetings Report Series No. 46A p. 93). The essential basis of the evaluation rested upon three aspects:

1. The long history of human use of carrageenan without known ill-effect.
2. Evidence that carrageenan is absorbed to a very small extent, if at all, when ingested by several animal species. (A distinction was not drawn between undegraded and degraded carrageenan in reviewing the available data.)
3. Available short- and long-term studies in animals. [These would not be considered adequate for safety evaluation by modern standards.]

Objectives of present work

The experiments described in this Report were intended to be an exploration of the distinctive biological properties of undegraded and degraded carrageenans as a preliminary phase to more formal and intensive study of these materials.

The fact that so much of the work done recently involved administration of carrageenan in the drinking water made it necessary to initiate work

along the same lines, even though it was obvious that this approach is unsuitable for formal studies intended to establish the safety-in-use of undegraded carrageenan as a food additive.

This preliminary phase of the work has now been substantially completed and the results are presented in this Report.

MATERIALS AND METHODS

Carrageenan solutions

Two forms of carrageenan were studied: a native, undegraded carrageenan (HMR) and a low-molecular degraded carrageenan (C16). For administration to animals, these materials were dissolved in sterile distilled water and the solutions administered as the sole source of drinking water. Fresh solutions were used every day. Samples of these solutions were subjected to microbiological examination; no contamination was found in a 24-hour period under the conditions prevailing in the animal facility. In addition, monkeys were given carrageenan by stomach tube. For this purpose, solutions of HMR, ranging from one to three percent concentration, were administered by intubation with a rubber catheter.

Diets and care of animals

The various animal species used in these studies were housed in temperature-controlled air conditioned quarters. All animals were individually caged in wire bottom cages which were regularly changed, and the drop pans were cleaned at least once weekly.

Food and drinking water (or carrageenan solution) were freely available at all times. Commercially formulated diets were provided ad libitum: Wayne Lab Blox, Guinea Pig Diet (Agway, Inc.), and Purina Monkey Chow were used for rats, guinea pigs, and monkeys respectively. Fresh water or carrageenan solution was provided daily.

Guinea Pigs

A. *Degraded Carrageenan (C16)*

Series 1. Ten male and ten female guinea pigs were given a 5% solution of C16 in sterile distilled water as their sole source of drinking water.

An additional group of five guinea pigs of each sex which were given sterile distilled water, served as controls. Body weights were obtained weekly. Stools were observed for consistency and were checked for occult blood by means of hematest tablets.

Guinea pigs that died were autopsied and examined grossly. Of the remainder, two of each sex were sacrificed on day 15, one of each sex on day 33, and the survivors on day 36. Autopsies and gross examinations were carried out; the intestinal tract was inspected with great care after rinsing away the luminal contents; the mucosal and serosal surfaces were examined by transmitted light. Numerous specimens of colon and cecum were fixed in 10% buffered formalin solution, and others in Zenker's solution with formalin. Sections of the colon and cecum were stained with hematoxylin and eosin, PAS, toluidine blue, or by Perls' iron procedure.

Series 2. A total of 33 female guinea pigs (average body weight 36.5 g) was used in this study: 25 in the experimental group and 8 in the control group. The experimental group comprised 8 animals given 0.02% C16 solution in place of drinking water, 8 animals given 0.2% C16 and 9 given 2.0% C16. The control animals received sterile distilled water. Administration of 2.0% solution was discontinued at two weeks.

Body weights were recorded two or three times weekly for the first three weeks and weekly thereafter. Fluid consumption was recorded daily. Stools were checked for consistency and occult blood.

Four guinea pigs were sacrificed from the 2.0% group between 14 and 26 days and guinea pigs that died were also autopsied.

B. *Undegraded carrageenan (HMR)*

Five male and ten female guinea pigs were given 1% HMR solution as drinking water and have now been on experiment for almost a year.

Body weights were recorded weekly. Stools were checked for consistency and occult blood.

For histologic studies, 2 males and 3 females were sacrificed at two months, 1 female at 3-1/2 months, 1 male at 7 months, and 1 female and 1 male at 8 months after the start of the experiment.

Rats

A. *Degraded Carrageenan (C16)*

Ten rats of each sex were given 5% solution of C16 as drinking water. Five of each sex given water served as the control group. The experiment is still in progress.

Body weights were recorded weekly. Stools were checked for consistency and for occult blood by means of hematest tablets.

Of the original 20 rats, groups of 2 male and 2 female rats were sacrificed at 1 month and at 3 months for histologic examination. In addition, 1 female was sacrificed at 8 months. Hematologic studies were carried out at 2 weeks and at 7 months.

B. *Undegraded carrageenan (HMR)*

Ten male and ten female rats were given 1% solution of HMR as drinking water. Records of fluid consumption were kept daily and body weights were recorded weekly. Stools were examined for consistency and occult blood.

Hematologic studies were done at four months. One male and one female from the HMR and control groups were sacrificed at five months. In addition two of each sex given the 1% HMR solution were sacrificed at six months. Sections of the gastrointestinal tract were examined after staining with hematoxylin and eosin.

Gerbils

A. *Degraded and undegraded carrageenan (C16, HMR)*

Two groups of five male gerbils each were given diets containing either 5% C16 or 5% HMR. A similar group was given stock diet.

Body weights and food consumption were recorded weekly. Stools were examined for consistency and occult blood. To date (six months) none of the gerbils have been sacrificed and they continue to consume the carrageenan-containing diets.

Monkeys

The overall plan of experiments with monkeys is shown in Table 1. A total of 20 young monkeys were used in this study with equal numbers of each sex in each group: 4 were controls that received plain drinking water. The remainder received either C16 or HMR in their drinking water. Ten monkeys were given C16; 6 received 2% C16, 2 1% C16 and 2 0.5% C16. In addition, 6 monkeys received 1% HMR. The duration of the treatment and recovery periods is indicated in Table 1.

Dose-ranging studies with HMR

Two monkeys from the 1% HMR group were killed at the end of 7 and 11 weeks respectively. At the end of 11 weeks, the remaining 4 animals were taken off carrageenan. After observation over a period of 11 weeks' recovery, these monkeys (#1003, 1004, 1034, 1035) were used for dose-range studies with HMR given by stomach tube. Increasing doses of HMR starting with 50 mg/kg through 1250 mg/kg were given to these animals over a period of thirty-three days.

Treatment at the highest dose was continued for 51 additional days, before the animals were killed for histological examination. For the

50 mg/kg dose a 1% solution of HMR (10 mg/ml) was given once daily. The volume of each dose was calculated for each monkey on the basis of body weight. The HMR solution was followed by 20 ml of a food slurry (a homogenate of canned monkey diet and water). As the dosage increased the concentration was raised to 2% and finally to 3%. The 1250 mg/kg dose was divided in half with one portion given in the morning and the second in the afternoon, each followed by 20 ml of food slurry.

Recovery studies: C16 monkeys

Two of the 6 monkeys (#1024 and 1017) receiving 2% C16 were killed at 7 and 11 weeks. The remaining four (#1006, 1009, 1021 and 1022) were allowed to recover for a period of between 140 and 168 days and were then autopsied for histological examination.

Autopsy and other procedures

The animals were killed by an overdose of barbiturates and autopsied immediately. All 3 body cavities were opened, their contents inspected *in situ* and after the removal of organs, they were reinspected (when feasible on cut surfaces). The large intestine was opened, the contents gently removed and the inner and outer surfaces examined using a magnifying lens and transmitted light. Various samples from cecum, all 3 parts of the colon and the rectum were placed on cork disks to avoid curling and fixed in 10% buffered formalin solution. Adjacent samples were fixed in Zenker's solution. In addition, representative samples from heart, trachea, lungs, esophagus, stomach, duodenum, small intestine, liver, gallbladder, pancreas, kidneys, urinary bladder, gonads, uterus, vagina, epididymis, pituitary, thyroid, adrenal, bone marrow, spleen, thymus, regional lymph nodes, brain, cerebellum, medulla oblongata, spinal cord and eyes were fixed also in 10% buffered formalin solution or in Zenker's solution with formalin.

Paraffin sections 6μ thick were stained with hematoxylin and eosin. All blocks from cecum, ileocolic valve, colon and adjacent lymph nodes were sectioned at 6 adjacent levels and stained by a modified trichrome method and with hematoxylin and eosin.

Standard methods were used in counting, staining and identifying the formed elements in the blood. For detecting occult blood in feces, hematest tablets were used, and the tests performed at least 3 times a week.

RESULTS

Section I. DEGRADED CARRAGEENAN (C16)

A. Guinea pigs

Guinea pigs given the 5% solution of C16 steadily lost weight (Table 2). Fluid intake, which was less than that of the control animals, averaged approximately 59 ml per day (Table 3). From this figure a daily intake of approximately 2.5 g (6-8 g/kg) of carrageenan was calculated.

Stools were soft within 24 hr and at 48 hr most were fluid in consistency. Occult fecal blood was detected in three animals within two days and in half of the guinea pigs within two weeks (Table 4).

Two females died at 9 days; at autopsy hemorrhagic ceca were observed in both. A total of 5 males and 4 females died during the course of treatment and one of each sex became moribund and was sacrificed after 33 days.

Pertinent gross observations included multiple petechiae in the cecum with scattered pale nodules in the cecal mucosa. Histologically, the submucosa was hyperemic and edematous. Focally there were dense accumulations of macrophages and fibroblasts in the lamina propria and submucosa. The macrophages contained iron positive pigment (Perls' method), were PAS positive and metachromatic with toluidine blue. Some scattered ulcerations of the epithelium were also seen, associated with an acute inflammatory response.

In the colon, there was an increase in the number of macrophages in the lamina propria and these cells also contained hemosiderin. The mesenteric and cecal lymphoid tissue was hyperplastic.

In the second study, guinea pigs in the control, 0.02%, and 0.2% groups remained normal in appearance and behavior. Food consumption and growth (Table 5) were normal. Stools have remained normal and were consistently

negative for occult blood (Table 6) throughout the 11 weeks of the study. On the other hand, guinea pigs given the 2% solution rapidly lost weight. Stools became soft within 24 hr and were semi-fluid in most animals thereafter. The stools were positive for occult blood within two days. Food consumpsion declined and the animals became weak and inactive. One died on the thirteenth day, and in spite of replacement of the 2.0% solution by plain water two more died within the next six days. One guinea pig developed a rectal prolapse and was sacrificed on the sixteenth day. Four were sacrificed for examination between the fourteenth and sixteenth days. The eighth pig died at 4 weeks as a result of an intercurrent infection. The ninth pig recovered, gained weight and appears completely normal.

Fluid consumption was similar in all groups, averaging approximately 60 ml/day (Table 7). Initial dosage was approximately 30, 300, and 3300 mg/kg per day in the 0.02%, 0.2%, and 2.0% groups, respectively.

In guinea pigs from the 2.0% group that died or were sacrificed between the thirteenth and sixteenth day, erosions of the cecum and/or the colon were observed in 4 out of 6 animals. Small pale nodules were observed in the wall of the cecum in 5 animals. Two animals that died were unsuitable for histologic examination because of advanced autolysis.

Histologic studies are in progress.

B. Rats

Food consumption and growth of female rats given 5% solution of C16 were normal (Table 8). Growth of the males, on the other hand, was less than that of the male controls. At nine months, the average body weight of the males given C16 was 18% less than that of the male controls.

Fluid intake of rats given 5% C16 was increased as compared with that of the control rats and the difference became greater as the study progressed (Table 9). Initial dosage was approximately 9.7 g/kg in males and 9 g/kg in females. By 14 weeks, because of the increased body weight of the male rats, the dosage declined in the male rats to approximately 6.4 g/kg. Thereafter the dosage of carrageenan remained reasonably constant at 6 to 7 g/kg/day. In the female rats, on the other hand, the daily intake of carrageenan remained constant at 9-10 g/kg/day throughout the 42 weeks.

Soft stools were seen within 24 hr. Thereafter consistency was variable, ranging from soft to semi-fluid. Occult blood was observed in some rats within one week and in most rats within four weeks. The presence of blood was occasionally observed in the stool (Table 10).

No alteration in erythrocyte count, hemoglobin or hematocrit was observed at 2 or 7 months (Table 11).

Other than a suggestion of hyperemia, no significant alteration has been observed grossly or microscopically in the gastrointestinal tract of the treated rats. No ulceration was found, nor was there any iron-positive or metachromatic material within macrophages in the lamina propria in those rats sacrificed at 1 or 3 months. One rat died after approximately 10 months of administration. Several small ulcers were found in the distal colon of this rat. Death had resulted from a severe respiratory infection.

C. *Monkeys*

General Condition

Monkeys given 2% C16 did not gain weight (Table 12). There was an immediate change in the character of the stools, which became loose, watery and unformed, and remained in this state as long as C16 was administered.

In addition, after 2-3 weeks, melena appeared, with occasional discharge of blood and mucus. By 10 weeks, two of the monkeys (#1006 and 1017) became ill and dehydrated.

Carrageenan was withdrawn and plain drinking water restored. Monkeys #1006 and 1017 received ampicillin (50 mg/kg intravenously) for two days, together with Electrolyte Solution R (10 ml intraperitoneally). There was slight improvement, but one of the animals (#1017) was sacrificed for histologic examination. The other was permitted to recover. After the remaining animals were restored to plain drinking water, their stools remained loose and watery for 4 weeks. Later the stools varied from watery and unformed to soft but formed stools. Two monkeys (#1021 and 1022) have had loose watery stools for almost 5 months after withdrawal of carrageenan. All four monkeys in this group, however, started gaining weight and appeared healthy (Table 13). They were sacrificed after 6 months of the recovery period and histopathologic examination is in progress.

Monkeys given drinking water containing lower levels of C16 (1% or 0.5%) gained weight (Table 14) and were generally in much better condition than those receiving 2% C16.

The record of occult blood tests in feces (hematest) is given in Table 15-17. Tests for occult blood were occasionally positive in the stools of control monkeys. The first (#1022) of the 4 monkeys given 2% C16 began to pass blood 6 days after treatment. The other animals on C16 showed occult blood in the feces on the nineteenth day. Thereafter animals receiving 2% and 1% C16 appeared to pass blood continuously, while the two monkeys on 0.5% C16 had a lower frequency of positive occult blood in the feces (Table 17). After withdrawal of carrageenan, the hematests remained positive for 10 weeks, after which there was progressive recovery (Table 16).

Daily water consumption was approximately 400 ml in controls and all test groups except the group on 2% C16, which ranged in weekly averages from 246-396 ml (Table 18). Approximate daily intakes of carrageenan were calculated to be as follows: 2% C16, 2.93 g/kg; 1% C16, 1.38 g/kg; and 0.5% C16, 0.70 g/kg.

Hematological data on the entire experiments with C16 in monkeys are presented in Table 19. The picture is consistently one of a fall in the number of erythrocytes and in the hematocrit as well as a less regular reduction in hemoglobin following prolonged intake of C16, with partial or full restoration to normal following a period of recovery.

Gross and Histopathology of Monkeys

A. 2% C16

Two monkeys (#1024 and #1017) were examined. The following organs were grossly and microscopically normal: heart, trachea, submaxillary glands, esophagus, stomach, small intestine, pancreas, kidney, urinary bladder, pituitary, thyroid, parathyroid, bone marrow, spleen, thymus, brain, cerebellum, medulla oblongata, spinal cord and eye.

Female #1024 (2% C16 for 7 weeks)

The gallbladder, ovary and uterus were normal in this animal.

Lung: Gross: a few mite cysts.

Microscopic: capillary hyperemia with some dark brown or black pigment deposits.

Liver: Gross: normal.

Microscopic: slightly distended sinusoids: increased number of granulocytes and enlarged Kupffer cells.

Colon: Gross: normal.

Microscopic: one small focal hemorrhage was present under a small area of thinned epithelium, which was covered with mucus and contained several erythrocytes. In the same section an additional area of thinned epithelium occurred. Infiltration with macrophages mixed with a few leukocytes was a striking feature in the tunica propria.

Lymph nodes: Gross: normal.

Microscopic: slight hyperplasia, with foamy macrophages.

Male #1017 (2% C16 for 11 weeks)

Lung: Gross: yellow spots.

Microscopic: small fibrotic granulomas with dark green pigment near one bronchus and in the immediate vicinity of small arteries.

Gallbladder: Gross: normal.

Microscopic: edema in submucosa.

Cecum: Gross: one large nodule in the mesentery. Cecal lumen filled with liquid stool.

Microscopic: slight edema and increased number of macrophages with minor capillary hyperemia.

Ileocolic valve:

Gross: normal except for some hyperemia.

Microscopic: two abscesses under the mucosa with central necroses containing fibrin and some fresh hemorrhages surrounded by loose connective tissue, containing multinucleated giant cells, macrophages, and fibroblasts. One of these abscesses was connected with the lumen by a deep ulceration of the mucosa. The colonic mucosa showed multiple hemorrhages, edema and a moderate increase in macrophages.

Colon: Gross: multiple hemorrhages throughout the entire colon with the exception of the rectum. These hemorrhages appeared to be covered with clotted blood that could not be removed from the mucosal surface by rinsing with saline.

Microscopic: (1) *10 cm aboral from ileocolic valve*: superficial erosions of the mucosa interspersed with multiple crypt abscesses that were seen in all layers of the remaining mucosa. The tunica propria was edematous, hyperemic and in some areas hemorrhagic. (2) *35 cm aboral from ileocolic valve*: one superficial erosion and one small ulcer were seen. The erosion was similar to the lesion as described under (1). The ulcer reached into the muscularis mucosae which was heavily infiltrated with macrophages and fibroblasts. In addition several crypt abscesses containing leucocytic debris. (3) *55 cm aboral from ileocolic valve*: capillary hyperemia, small hemorrhages and edema of the submucosa. Several crypt abscesses containing cellular debris were present in the mucosa.

Mesenteric lymph nodes:

Gross: the mesentery of the colon had a chain of prominent lymph nodes.

Microscopic: the regional lymph nodes near the cecum, colon and one node close to the head of the pancreas were slightly hyperplastic; however, the most predominant change was a distention of the marginal and central sinus containing large numbers of reticular cells with large, clear, vacuolated cytoplasm. In some instances an eosinophilic edema was also present.

Liver: Gross: cut surface yellow.

Microscopic: a moderate increase in the number of leucocytes was present in the sinusoids, sometimes found in focal aggregates of 5-20 individual cells. The Kupffer cells were slightly enlarged, with elongated nuclei and faintly eosinophilic granular cytoplasm.

Kidney: Gross: normal.

Microscopic: a few cysts lined with epithelium in cortex.

Testes: Gross: small.

Microscopic: immature, no signs of spermatogenesis.

Prostrate: Gross: small.

Microscopic: immature.

Adrenal: Gross: normal.

Microscopic: several areas of focal calcification.

B. 1% C16

Two monkeys (#1020 and #1005) were subjected to gross and microscopic examination and the following organs were found to be normal: heart, submaxillary glands, oesophagus, stomach, small intestine, gallbladder, rectum, pancreas, kidney, urinary bladder, thyroid, pituitary, bone marrow, spleen, thymus, brain, cerebellum, medulla oblongata, spinal cord and eye.

The lungs of both animals were infested by mites, and certain secondary changes were observed along with slight pigment deposition. In both, the liver was grossly normal and microscopic examination revealed prominent Kupffer cells, which were enlarged with granular cytoplasm.

Detailed histology of the gut, large intestine and lymph nodes of monkeys #1020 and #1005 is presented below:

Female #1020 (1% C16 for 14 weeks)

Cecum: Gross: normal.

Microscopic: a few focal intestial cells infiltrating into the submucosa.

Ileocolic valve:

Gross: slight hyperemia.

Microscopic: capillary hyperemia and edema of mucosa with diffuse or focal infiltrations of macrophages. One crypt abscess contained cellular debris. A relatively large number of hyperplastic lymph nodes were found in the submucosa.

Colon: (1) *10 cm below ileocolic valve.*

Gross: deep crypts, some edema.

Microscopic: slight capillary hyperemia and edema of mucosa. One small crypt abscess in one area with thin mucosa. Relatively large numbers of submucosal hyperplastic lymph nodes.

(2) *30 cm aboral from ileocolic valve.*

Gross: multiple hemorrhagic areas, largest diameter approximately 1-2 mm.

Microscopic: capillary hyperemia and focal intramucosal hemorrhages. A few small crypt abscesses, in addition to shallow erosions associated with an increased number of macrophages.

(3) *60 cm aboral from ileocolic valve.*

Gross: multiple hemorrhagic areas, largest diameter approximately 1-2 mm.

Microscopic: two small erosions of mucosa with different densities of macrophages.

Regional lymph nodes:

Gross: prominent glossy lymph nodes were seen thoughout the entire mesentery.

Microscopic: all regional lymph nodes examined were hyperplastic; there was distention of the marginal and especially of the central sinus, largely occupied with large reticulum cells containing foamy cytoplasm.

Ovary and uterus:

Gross: small and immature.

Microscopic: immature.

Adrenal: Gross: normal.

Microscopic: few colloid-containing cystic spaces in medulla.

Male #1005 (1% C16 for 14 weeks)

Cecum: Gross: normal.

Microscopic: slight capillary hyperemia.

Ileocolic valve:

Gross: normal.

Microscopic: one shallow erosion of mucosa. The underlying lymph node contained a large, centrally-located area of necrosis, presumably an old crypt abscess, since in some areas remnants of low cuboidal epithelium similar to epithelial linings of the crypt abscesses mentioned above were seen. In the base of some crypts were seen a large number of small, darkly-stained particles, both intracellular and extracellular. These are presumably parasites that were also found in regional lymph nodes.

Colon: (1) *10 cm aboral from ileocolic valve.*

Gross: slight edema and hyperemia.

Microscopic: slight edema, hyperemia and infiltration of the mucosa with macrophages. In two hyperplastic lymph nodes located below the mucosa, cross-sections were seen of parasites surrounded by pus and young granulation tissue with multinucleated giant cells and collagen fibers.

(2) *35 cm aboral from ileocolic valve.*

Gross: focal hyperemia and hemorrhages with the largest diameter approximately 1 mm.

Microscopic: slight hyperemia and occasional apical hemorrhages in the mucosa. One fresh erosion, some focal infiltration of mucosa with macrophages.

Regional lymph nodes: Two types of lymph nodes were found, especially around the cecum and the proximal part of the ascending colon. The first type was enlarged and white; microscopically, there was moderate hyperplasia with some distention of marginal and central sinuses containing large numbers of reticular cells with foamy cytoplasm. The second type of lymph node was black and glossy. Microscopically, a central lymphadenitis was present, containing a cross-section of a parasite at the margin of the lesion. Slight fibrosis and young connective tissue with multinucleated giant cells surrounded the lesion.

Kidneys: Gross: normal.

Microscopic: a few foci of cortical round cell infiltration.

Testes: Gross: small, approximately 20 mm. largest diameter.

Microscopic: immature.

Adrenal: Gross: normal.

Microscopic: some accessory nodules, with cell patterns similar to zona glomerulosa within and outside the capsule; two areas of focal calcification.

Brain (motor cortex):
Gross: normal.

Microscopic: several periarteriolar edematous areas containing a few red cells.

C. 0.5% C16

In two monkeys (#1023 and #1008), the following organs were grossly and microscopically normal: heart, lungs, submaxillary gland, stomach, small intestine, gallbladder, kidneys, urinary bladder, uterus, pituitary, thyroid, bone marrow, spleen, thymus, brain, cerebellum, medulla oblongata, spinal cord and eye.

Female #1023 (0.5% C16 for 14 weeks)

Cecum: Gross: normal.

Microscopic: some focal round cell infiltration.

Ileocolic valve:
Gross: normal and pale.

Microscopic: slight round cell infiltrations mixed with macrophages; three deep crypts with rather shallow epithelium and containing some epithelial debris. Slight hyperplasia of submucosal lymph nodes.

Colon: (1) *20 cm aboral from ileocolic valve.*

Gross: normal.

Microscopic: slight hyperemia and minute hemorrhages in the mucosa. A fresh shallow erosion of the mucosa over a slightly hyperplastic lymph node.

(2) *50 cm aboral from ileocolic valve.*

Gross: several hemorrhagic areas, largest diameter approximately 3 mm.

Microscopic: capillary hyperemia and some mucosal hemorrhages. One shallow erosion reaching to the lamina propria.

Regional lymph nodes:
Gross: normal.

Microscopic: very slight hyperplasia with proliferation of reticular cells which were enlarged and showed foamy cytoplasm.

Kidney: Gross: normal.

Microscopic: a few foci of interstitial round cell infiltration.

Ovary: Gross: immature.

Microscopic: immature, no corpora lutea.

Adrenal: Gross: normal.

Microscopic: two small focal calcifications at cortical medullary border.

Male #1008 (0.5% C16 for 14 weeks)

Cecum: Gross: normal.

Microscopic: normal.

Liver: Gross: normal.

Microscopic: some focal round cell infiltrations.

Gallbladder: Gross: normal.

Microscopic: few focal round cell infiltrations in submucosa.

Ileocolic valve:
Gross: normal.

Microscopic: few areas of capillary hyperemia and slight edema of submucosa.

Colon: Gross: normal.

Microscopic: (1) *20 and 60 cm aboral from ileocolic valve.* Occasionally some capillary hyperemia.

Kidney: Gross: normal.

Microscopic: normal.

Summary of Histopathology

The most significant changes were seen in the large intestine. Monkey #1024 was least affected, because this animal received 2% C16 in drinking water for only 7 weeks. More pronounced changes in the colon were found in monkey #1017 that had received 2% C16 for 11 weeks. Hemorrhages, erosions, ulcers, crypt abscesses and submucosal abscesses associated with an increased number of macrophages in the tunica propria were the most characteristic findings in the colon.

The four animals that received the lower dose levels of C16 (1% and 0.5%) in drinking water for 14 weeks yielded similar findings, except that ulcers were not seen in these animals. The mucosal hemorrhages, taken together with the epithelial defects, can account for the blood loss documented earlier. The crypt abscesses represent an inflammatory response caused by a focal decreased resistance of the mucosal lining. The diffuse increase in the number of macrophages was usually not associated with focal changes in the mucosa, and might be regarded as an almost independent reaction of the colonic mucosa.

All regional lymph nodes, especially those in the vicinity of the cecum and the ileocolic valve were hyperplastic and contained in all instances variable numbers of enlarged reticular cells. These cells showed cytoplasmic changes indicative of the uptake of some foreign material and could therefore be classified as macrophages.

The Kupffer cells in the livers from the four animals receiving the two highest concentrations of C16 (2% and 1%) in drinking water were enlarged, showing a granular cytoplasm also indicative of the uptake of a foreign substance. This phenomenon was absent in the two animals receiving 0.5% C16.

All other changes listed above were either minor in degree or known as spontaneous diseases in rhesus monkeys. Their incidence and degree did not show any relationship to treatment.

In summary, prolonged administration of all three levels of C16 in drinking water produced adverse effects in the colon and changes in the regional lymph nodes.

RESULTS

Section II. UNDEGRADED CARRAGEENAN (HMR)

A. Guinea pigs

The guinea pigs given 1% HMR in drinking water remained normal in appearance and behavior. Stools remained normal and were negative for occult blood. Growth up to 3 months was comparable to that of the controls (Table 20). The small number of animals in the HMR group thereafter prevented any valid comparison.

Fluid consumption ranged from 41-75 ml/day (Table 21). The approximate calculated intake of HMR was therefore 0.8-1.1 g/kg/day.

No gross abnormality was found at autopsy. Numerous macrophages containing hemosiderin (Perls' positive) were observed in the lamina propria in sections of the cecum. Material in the macrophages was PAS positive, but was not metachromatic with toluidine blue. The colon was histologically normal.

B. Rats

Food consumption and growth of rats on 1% HMR was normal (Table 22). Fluid consumption ranged from 35-62 ml in males and 29-60 ml in females (Table 23). Initial intake of HMR was thus approximately 1.3 and 1.8 g/kg/day in males and females respectively, and declined over six months to 600-900 mg/kg/day. Although stools were somewhat softer than those of the control rats, they were well formed and consistently negative for occult blood. Hematological indices taken from 5 males and 5 females given HMR were normal after 4 months (Table 24).

In one male and one female rat given 1% HMR and sacrificed at 5 months, areas were found in the cecum where the mucosa was reduced to a single layer of columnar cells. The underlying lamina propria was infiltrated

with lymphocytes and macrophages. In such areas the muscularis mucosae was absent, and the submucosa was occupied by a large lymph node. Similar areas were not observed in those rats sacrificed at 6 months. However, an area with nearly identical features was observed in one of the control rats sacrificed at 5 months.

In the remaining animals, gross and microscopic examination of stomach, small intestine, cecum and colon disclosed no changes attributable to the administration of HMR.

C. *Gerbils (HMR and C16)*

Since the results of administration of HMR and C16 to gerbils were essentially the same, these groups will be discussed together. The gerbils given diets containing 5% C16 or 5% HMR were normal in appearance and behavior. Food consumption in both groups given carrageenan was slightly higher than that of the control group (Table 25). Increase in body weight was also slightly greater in the groups given carrageenan than in the control group (Table 26).

Intake of carrageenan was approximately 3550 mg/kg/day and 4200 mg/kg/day in the C16 and HMR groups respectively. At 23 weeks these figures were 2000 mg/kg/day in both groups.

The stools in both groups were well formed, but were slightly more moist than those in the control group. Hematests for occult blood were consistently negative.

One gerbil in the HMR group was accidently killed in the 5th week. No gross abnormalities were found at autopsy. The experiment is still in progress.

D. Monkeys

General condition

Six monkeys were given 1% aqueous solution of HMR as the sole source of drinking water. Their daily consumption of fluid was approximately 400 ml (Table 27), so that the calculated intake of HMR was 1.3 g/kg/day. One monkey (#1026) was killed at 7 wk and the remaining monkeys continued on HMR for a total of 10 wk, when they were placed on plain drinking water. A second monkey (#1002) was killed at this time. All monkeys remained well and were completely normal clinically (Table 28). Except for the finding of occult blood, described below, no observation was made suggestive of an effect of administration of HMR.

After a recovery period exceeding 2 months, the remaining 4 monkeys were used in a dose-ranging experiment involving administration of HMR by stomach tube. The substantial quantities of HMR ingested did not appear to affect the animals adversely. They continued to gain weight (Table 29) and remained in good condition. Stools obtained from the HMR monkeys were usually well formed and normal in appearance during the duration of the study, except for a sporadic occurrence of soft or watery stool, which was pale in color with an oily coating on occasion. When HMR administration ceased, stools returned to normal color and consistency. The record of occult blood test results is shown in Table 30-31. The first positive test was observed in one animal (#1004) after 40 days of HMR administration (Table 30). Positive findings over a period of 1 week or less in females coincided with menstruation. During the recovery period following 72 days of treatment only occasional positive results were seen in test animals as well as in controls. In the dose-ranging experiment, despite the massive doses of HMR finally attained, only sporadic positive findings of occult blood resulted, more or less similar to those in controls (Table 31).

Despite this reassuring picture, the results of hematologic studies (Table 32) suggest a consistent fall in the number of circulating erythrocytes after 9 weeks of ingestion of 1% HMR in drinking water. The hemoglobin and hematocrit levels showed no such effect. The dose-ranging studies also left the hematologic indices substantially unaltered.

Gross and Histopathology of Monkeys

Two monkeys, 1 female (#1026) and 1 male (#1002) which had received 1% HMR for 7 and 11 weeks respectively were examined.

The heart, trachea, esophagus, stomach, small intestine, gallbladder, pancreas, pituitary, submaxillary gland, thyroid, parathyroid, adrenal, bone marrow, spleen, thymus, brain, cerebellum, spinal cord and eye of both animals were examined and found to be normal. In addition, the urinary bladder, uterus and vagina of the female, and the cecum and medulla oblongata of the male were found to be normal.

The lungs appeared normal grossly, but microscopic examination revealed pigmented granulomas, presumably of parasitic origin. Microscopic examination of the liver of the female revealed a few round cell infiltrations in some areas, an increased number of leucokytes in the sinusoids, a few enlarged Kupffer cells with elongated nuclei and slightly granular cytoplasm. The gallbladder of the male was in two parts with no communication between the two, but microscopic examination showed it to be normal. The kidney of the male appeared normal, although microscopic examination revealed a few focal lymphocytic interstitial infiltrations. The testes and epididymis appeared small and microscopic examination proved them to be immature with no signs of spermatogenesis. Microscopic examination of the peripancreatic lymph node showed focal eosinophilic edema.

Gross examination of the stomach of one male contained reddish-brown material with some brown flakes, which was thought to be swallowed blood from a freshly broken upper incisor.

In the colon of the female, some minute areas of capillary hyperemia appeared to be associated with compacted contents compressed into the rugae, and microscopic examination showed capillary hyperema and edema of mucosa.

The colon of the male had two trichobezoars 15 and 25 cm. aboral from the ileocolic valve and a stool of pasty consistency.

Dose-ranging studies with HMR

Examination of the remaining 4 monkeys (males #1003, 1004; females #1034, 1035) revealed no gross or microscopic tissue changes directly attributable to the administration of HMR. Special attention was given to the gastrointestinal tract, particularly the colon. At autopsy there was no indication of any change in the small intestine or colon. In one monkey (#1004) numerous dark brown nodules were found in the mesentery of the colon. Except for adhesions to the left lung in #1004, all thoracic and abdominal viscera appeared normal. In #1003 an abnormal bone growth of the left orbit resulted in compression of the left frontal lobe of the brain; histologically, there was thickened normal bone causing gliosis of the left frontal lobe.

The microscopic appearance of the gastrointestinal tract was essentially normal. The mucosa was intact and there were no unusual cell infiltrations indicative of an inflammatory response. Scattered foci of slight hyperemia of the submucosal vessels and occasionally of the mucosal capillaries were observed in the colon of #1004. The hard brown nodules observed in the mesentery of this monkey were found to be encapsulated abscesses containing cross-sections of unidentified parasites. Similar mesenteric abscesses were also found in #1035. The mesenteric lymph nodes in the other two monkeys were normal.

Slight alterations were found in the livers of three of the four monkeys. In two of them (#1034 and 1035) there was a diffuse infiltration of granulocytes in the sinusoids, together with a few small accumulations of mononuclear cells. The Kupffer cells and parenchymal cells were normal.

In one (#1004) slight to moderate swelling of the parenchymal cells was observed throughout the liver. The parenchymal cells were intact and there were no prominent morphological abnormalities.

Summing up the observation made, it is concluded that, despite the high doses of HMR to which these monkeys were exposed, no histopathologic change attributable to carrageenan was observed.

DISCUSSION

Issues that need to be resolved

It is useful at the outset to list as many as possible of the questions that arise from the study of the effects of carrageenan in animals. This Report of preliminary studies cannot be expected to resolve many of them, but an analysis of this sort does at least indicate the extent of the information gap that has been created by the published observations on carrageenan.

The issues that arise are as follows:

1. To what extent is the effect of carrageenan related to:
   a. administration in the drinking water, as distinct from customary routes of oral administration, namely by gavage or in the diet
   b. the total dose of carrageenan administered daily
2. To what extent is the effect specific for:
   a. carrageenan itself, as distinct from other sulfated polysaccharides
   b. an iota carrageenan, as distinct from kappa and lambda carrageenans
   c. carrageenans predominantly composed of moieties having weight average molecular weight approximately 20,000, as distinct from native carrageenans
   d. 'native' carrageenans containing a small proportion of low-molecular fraction, either naturally-occurring or produced during processing of food under acid conditions and high temperature
3. To what extent is the effect of carrageenan species specific:
   a. is the effect restricted to the guinea pig and rabbit
   b. is the effect restricted to "herbivorous" animals
   c. can the effect be elicited, under appropriate conditions, in any species of laboratory animal
   d. is the "effect" in fact the same in all species in which it is manifested

4. Is the effect of carrageenan mediated by:
   a. absorption through the gut wall
   b. pinocytosis into the intestinal epithelium
   c. uptake into subepithelial macrophages
   d. formation of a complex with food protein or with mucosal surface protein, which is then taken up into the intestinal epithelium
5. What part is played by the intestinal flora in the causation of the effects of carrageenan:
   a. by bringing about degradation (desulfation, hydrolysis) of the carrageenan, thus possibly facilitating absorption
   b. does carrageenan bring about alteration in the composition and/or activity of the intestinal flora
6. What part is played by immunological phenomena:
   a. is carrageenan, or its degradation products, or its complex with protein, antigenic
   b. is pre-existing sensitisation to some endogenous or exogenous sulfated polysaccharide responsible for cross-reaction with carrageenan or its degradation products; and is ulceration of the lower bowel a manifestation of this immunological response
7. What part is played by electrolyte imbalance:
   a. would carrageenan in the potassium form bring about the same effect
   b. would carrageenan complexed with protein, for instance milk protein, bring about the same effect
8. What is the relevance of the animal work to man:
   a. with respect to the use of C16 as a drug
   b. with respect to the use of IMR as a food additive
   c. with respect to any relationship whatever to human ulcerative colitis

Distinction between undegraded (HMR) and degraded (C16) carrageenans

In our view, much of the confusion created by contradictory statements in the literature stems largely from inadequate descriptions of the experiments performed and results obtained. To some degree also the uncertainties are compounded by a failure to distinguish clearly between the effects of degraded and undegraded carrageenans. In an effort to avoid problems of this sort, we shall begin by discussing the effects of C16 and of HMR separately.

DEGRADED CARRAGEENAN

The effect observed in guinea pigs

The original observations by Marcus and Watt (1969) and Watt and Marcus (1969, 1970) involved the administration of levels as high as 5% of degraded carrageenan (from *Chondrus crispus* and *Echeuma spinosum*) in the drinking water of guinea pigs. These studies have been summarised by Thayer (1970) and a full account has now been published by Watt and Marcus (1971).

In essence, what we are concerned with here is ulceration of the cecum, developing during the initial period of 20-25 days' administration of degraded carrageenan. Prolongation of this regimen to 30-45 days resulted in similar involvement of the colon and rectum. The critical features of the lesion were as follows: congestion of capillaries, edema of the mucous membrane, infiltration of inflammatory cells (macrophages, polymorphonuclear cells and lymphocytes); ulceration that was either focal or that extended into the muscularis mucosae; and the presence of crypt abscesses.

Sharratt *et al.* (1970) administered 1% degraded carrageenan (from *E. spinosum*) in the drinking water for 2-4 weeks and elicited multiple ulcers in the cecum of the guinea pig. According to these authors the sequence of events leading up to ulceration began with a striking macrophage response in the lamina propria, followed by a granulomatous inflammatory phase. Ulceration was accompanied by

infiltration of polymorphs, lymphocytes and plasma cells. It is noteworthy that no crypt abscess nor epithelial hyperplasia was observed.

Finally, in an unpublished report, Maillet has described the administration to guinea pigs of a 33% gel of degraded carrageenan (C16 from *E. spinosum*), given by stomach tube three times daily to a total daily dose of 2 g/kg. For purposes of comparison a 5% solution of C16 was administered as drinking water in two ways: one group of animals received the solution *ad libitum* (attaining daily intakes of C16 of 4g/kg), while another received a limited amount calculated to approximate a total daily intake of 2 g/kg C16. This interesting comparison proved most illuminating. Unlimited intake of the 5% C16 solution resulted in severe loss of weight, occult blood in the stools, cecal and colonic ulceration by day 30 in all the 15 animals, and death of 7 of these guinea pigs. Restriction of intake of 5% C16 solution to a dose of 2 g/kg/day also caused severe loss of weight and 20% deaths, but no positive occult blood tests and no intestinal lesions. Forced feeding of C16 3 times daily permitted normal weight gain; there was one animal with a positive hematest and one death, the cause of which is not specified. No ulceration of the intestine had developed after 30 days. Histologically, the groups in which intake of C16 was restricted merely showed inflammatory cell infiltration of the cecum and colon. The *ad libitum* group had, in addition, thinning and loss of epithelium, frequently edema of the mucous membrane and ulceration penetrating to and affecting the muscularis mucosae.

In a further unpublished study, Maillet followed the sequence of changes in the cecum after 1 month's treatment with 2 g C16/kg daily (33% C16 gel). One month after the start of treatment 2/10 animals had died and the remainder showed severe cecal, as well as scattered colonic, ulceration. In the group sacrificed after a further month (without treatment) there were 3/10 deaths and lesions localised to the cecum only (in 5/10 animals). After 2 months and 3 months without treatment the survivors (7/10 and 8/10 respectively) had occasional foci of

of granulation tissue surrounding regenerating glandular structures. Re-epithelialisation had occurred over these areas.

The experiments described in this Report confirm the susceptibility of the guinea pig to high doses (up to 8 g/kg) of 5% C16 carrageenan given in the drinking water. The lesions described have much in common with those reported by Sharratt et al. (1970). The lesions differ from the account given by Watt and Marcus (1969) in two important respects: the absence of crypt abscesses and of epithelia hyperplasia.

Watt and Marcus (1969) have been the only authors to report ulceration produced by carrageenan in rats and mice. Other workers could not confirm these observations (Sharratt *et al.*, 1970; Maillet et al., 1970). Our own experiments with rats elicited no gross nor histopathological changes in the intestine after administering 5% C16 carrageenan in the drinking water for up to 266 days. On the other hand, the record of occult blood tests (hematest) in the feces is clearly positive throughout, in comparison with almost invariably negative findings in untreated control groups (Table 10).

The limited accuracy of clinical tests for occult blood, such as hematest and hemastix, is well recognized (Ross and Gray, 1964). Moreover the feces of rodents, especially the rat, contain considerable quantities of protoporphyrin secreted by the Harderian glands. It has been suggested that fecal ferrous sulfide, exposed to acetic acid used in the benzidine test, forms ferrous acetate, which reacts with the protoporphyrin to form hematin (Salmon and Gellatly, 1970). These reservations notwithstanding, it seems clear that exposure of rats to C16 led to fairly consistent presence of occult blood in the feces. The limited hematological measurements carried out (Table 11) did not suggest that blood loss of this sort over a period of 7 months had influenced the peripheral blood.

Gerbils tolerated C16 even better than rats, without any apparent effect on growth or the nature of the stools.

Non-human primates

Sharratt et al. (1971) made passing reference to the fact that neither absorption of iron-labelled carrageenan, nor macrophage accumulation, nor ulceration had been observed in any part of the gastrointestinal tract of the squirrel monkey after treatment with degraded carrageenan. Details of dose or time of exposure were not provided.

In the experiments with rhesus monkeys reported here, the levels of degraded carrageenan in the drinking water were 2%, 1% or 0.5%. The duration of exposure to 2% C16 was as long as 11 weeks. The monkey killed at 7 weeks had minimal changes in the colon, despite the fact that occult blood had been found in its stools on two occasions. The next monkey was killed at 11 weeks, after a prolonged and fairly consistent series of positive tests for fecal occult blood. Focal ulceration of the cecum and colon was present, with crypt abscesses, usually limited to the ulcerated areas.

The remaining 4 monkeys that had been subjected to 2% C16 for 10 weeks were permitted to recover for 20-24 weeks, during which time occult blood continued to be observed in the stools, particularly in 3 out of the 4 animals. At autopsy after 20 weeks in one monkey and 24 weeks in the rest, all that could be noted grossly was some prominence of lymph nodes adjacent to the cecum and colon. In all other respects the intestine was normal. Detailed histopathological study of the tissues from these animals is in progress.

A clear picture of the dose-response relationships in monkeys given the three levels of C16 in drinking water emerges from consideration of Table 33: The detailed comparison of histopathological changes seen in the cecum and colon reveals the absence of ulceration at 1% and 0.5% C16 and the fact that minimal changes are present in the cecum as opposed to the colon. In the colon, erosion of epithelium and the presence of crypt abscesses were the most notable features.

The rectum was not affected in any animal examined.

Despite the small numbers of animals on which Table 33 is based, the consistency of the changes in relation to dose provides confidence in the general conclusions that may be drawn from this work. The doses to which the monkeys were exposed in the three groups were approximately 2.9, 1.4, and 0.7g/kg/day. It seems reasonable to assume on the basis of our results that administration of doses more closely approximating those to which man is exposed would produce no detectable change in the integrity of the gastrointestinal epithelium.

UNDEGRADED CARRAGEENAN

Watt and Marcus (1969) administered 1% aqueous solution of undegraded carrageenan derived from *E. spinosum* to guinea pigs for periods of from 23-30 days. The overall incidence of ulceration was 80%. More recently Sharatt *et al.* (1970) reported multiple cecal ulcerations produced in guinea pigs by administration for 2-4 weeks of 5% native carrageenan (extracted from *E. spinosum*) in the diet.

In the present work, administration of 1% HMR in drinking water to guinea pigs for as long as 1 year has had no ill effect on these animals. Growth has been normal. Stools were well-formed and have remained negative for occult blood. Histologic examination of the cecum and colon obtained from animals killed at 2, 7 and 8 months has revealed no abnormality. Macrophages were PAS positive and some of them contained hemosiderin, but they did not stain metachromatically with toluidine blue.

Maillet et al. (1970) and Sharratt et al. (1970) administered 5% native carrageenan to rats for 2-4 weeks in the diet. Both groups did not find any ulceration or other change in the cecum and colon of rats. Results obtained in this Institute confirm these observations. Male and female rats given 1% HMR

as the sole source of drinking water for as long as 4 months suffered no apparent ill effect. Growth of the rats was observed to be normal. Stools were somewhat softer than those of controls, but were well formed and consistently negative for occult blood. Gross and microscopic examination of the cecum and colon disclosed no change attributable to the administration of HMR.

A review of the available literature makes no mention of monkeys being used as experimental animals with undegraded carrageenan. In the present work 6 young rhesus monkeys (3 males and 3 females) were given 1% HMR in the drinking water. The general condition of these monkeys was good; if anything, weight gain was better than in the controls. Making allowance for menstruation, we conclude that the incidence of positive tests for occult blood in the stools was inconsistent and did not differ significantly from the sporadic positive results in the controls. Certainly the overall pattern was very different from that seen in the C16 monkeys. Two monkeys (one female and one male) that had received carrageenan for 7 and 11 weeks were autopsied and examination of the large intestine showed this to be normal except for some small areas of capillary hyperemia and edema of the mucous membrane in the female (possibly associated with inspisated bowel contents between the rugae), and trichobezoars in the region of the ileocolic valve.

The remaining 4 monkeys were off treatment for 11 weeks, during which time the incidence of fecal occult blood was similar to that of the controls. The same monkeys then received HMR at doses attaining a value of 1250 mg/kg, given by stomach tube daily, the experiment extending over a period of 84 days. At autopsy the large intestine was completely normal in every animal, and histologic examination reveals no changes anywhere in the gastrointestinal tract.

Some General Conclusions

Although the experiments reported here are not, and were not intended to be, conclusive in themselves, they do establish a clear distinction between the effects of degraded C16 carrageenan and native HMR carrageenan. What the source of this difference might be, is still an open question.

With regard to the effects of C16, there are evidently species differences to be accounted for. The dose-response relationship established in the rhesus monkey, a susceptible species, holds promise that further work will delineate unequivocally a no-effect dose as far as damage to the intestine is concerned. The reasons for the susceptibility of the guinea pig, the absence of histopathological change in the intestine of the rat, and the total resistance of the gerbil (to C16 in the diet) are all questions worthy of further investigation.

HMR has proved singularly free from any capacity to injure the various species of animals studied, even under extreme conditions of exposure. Nevertheless, the tests carried out do not constitute a basis for safety evaluation. The widespread and increasing use of native carrageenans as food additives, as well as the complexities of the issues involved in this group of compounds, demand a very thorough and up-to-date approach to the establishment of their safety.

Table 1. *Summary of nature and duration of exposure of rhesus monkeys (Macaca mulatta) to carrageenan*

Group	Monkey No.	Sex	Time (wk)	
			On test	Recovery
Control	1010	M	–	–
	1016	M	–	–
	1028	F	–	–
	1029	F	–	–
2% C16	1006	M	10	24
	1009	M	10	24
	1017	M	11	0
	1021	F	10	20
	1022	F	10	24
	1024	F	7	0
1% C16	1005	M	14	0
	1020	F	14	0
0.5% C16	1008	M	14	0
	1023	F	14	0
1% HMR	1002	M	11	0
	1003	M	10	11
	1004	M	10	11
	1026	F	7	0
	1034	F	10	11
	1035	F	10	11
HMR Dose-ranging	1003	M	12	0
	1004	M	12	0
	1034	F	12	0
	1035	F	12	0

Table 2. *Individual weekly body weights (g) of guinea pigs given 5% C16 in drinking water*

Sex	Group	Animal	Time (wk) 0	1	2	3	4	5
Female	Control	1	400	432	514	536	608	615
		2	430	471	526	538	602	572
		3	409	420	261	Killed at 15 days		
		4	439	467	504	544	576	630
		5	414	438	442	452	443	407
Male	Control	1	450	459	451	Killed at 15 days		
		2	391	426	489	511	571	607
		3	397	427	461	479	505	495
		4	445	414	450	384	383	358
		5	403	425	457	496	566	607
Female	5% C16	6	365	303	Died at 9 days			
		7	410	312	290	Killed at 15 days		
		8	411	376	359	348	332	328
		9	467	437	368	343	290	Died at 32 days
		10	533	417	382	364	Died at 24 days	
		11	427	389	393	356	360	334
		12	419	353	300	Killed at 15 days		
		13	421	360	331	326	295	Killed at 32 days
		14	338	279	Died at 9 days			
		15	408	382	368	374	336	306
Male	5% C16	6	394	360	353	Killed at 15 days		
		7	415	377	384	353	335	Killed at 35 days
		8	413	386	328	Killed at 15 days		
		9	441	417	328	319	290	Killed at 32 days
		10	438	371	319	264	275	Died at 35 days
		11	458	398	372	379	306	Killed at 36 days
		12	385	349	318	Died at 20 days		
		13	416	381	365	313	Died at 27 days	
		14	435	409	384	348	Died at 28 days	
		15	389	380	397	327	341	Died at 34 days

Table 3. *Weekly average of measured daily intake of fluid by guinea pigs given 5% C16 in drinking water*

Week	Ave. daily intake of fluid (ml)	
	Controls	5% C16
1	86	56
2	90	57
3	96	59
4	96	57
5	98	66

Table 4. *Record of occult blood tests (Hematest) on feces of guinea pigs given 5% C16 Carrageenan in drinking water*

Sex	Group	Animal	Time (days) 2	8	14	36	
Female	Control	1	-	-	-	-	Killed 36 days
		2	-	-	-	-	Killed 36 days
		3	-	-	-	Killed 15 days	
		4	-	-	-	-	
		5	-	-	-	-	
Male	Control	1	-	-	-	Killed 15 days	
		2	-	-	-	-	Killed 36 days
		3	-	-	-	-	Killed 36 days
		4	-	-	-	-	
		5	-	-	-	-	
Female	5% C16	6	-	+	Died 9 days		
		7	-	-	+	Killed 15 days	
		8	+	-	+	+	Killed 36 days
		9	-	-	-	Died 32 days	
		10	-	-	+	Died 24 days	
		11	+	-	+	-	Killed 36 days
		12	-	-	-	Killed 15 days	
		13	-	-	-	Killed 33 days	
		14	+	+	Died 9 days		
		15	-	-	+	+	Killed 36 days
Male	5% C16	6	-	-	-	Killed 15 days	
		7	-	-	-	+	Killed 36 days
		8	-	-	+	Killed 15 days	
		9	-	-	-	Killed 33 days	
		10	-	-	+	Died 32 days	
		11	-	-	-	+	Killed 36 days
		12	-	-	+	Died 20 days	
		13	-	-	-	Died 27 days	
		14	-	+	-	Died 28 days	
		15	-	-	+	Died 35 days	

Table 5. *Average weekly body weights (g) of female guinea pigs given 0.02%, 0.2%, or 2.0% C16 Carrageenan in drinking water*

Group		Average weekly body weight (g)											
		Weeks											
		0	1	2	3	4	5	6	7	8	9	10	11
Control	Wt.	366	400	427	460	492	538	562	589	617	630	640	634
	No.	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)
0.02% C16	Wt.	366	401	440	478	482	528	543	571	589	604	628	627
	No.	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)
0.2% C16	Wt.	365	399	431	461	512	551	591	606	639	650	651	675
	No.	(8)	(8)	(8)	(8)	(7)	(7)	(7)	(7)	(7)	(7)	(7)	(7)
2.0% C16	Wt.	365	323	289*	373	414	547	580	622	576	645	655	687
	No.	(9)	(8)	(3)	(2)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)

* Administration of 2% stopped at 2 weeks

Table 6. Record of occult blood tests (Hematest) on feces of guinea pigs given 0.02%, 0.2% or 2.0% C16 Carrageenan in drinking water

Sex	Group	Animal No.	Time (days) 2	7	9	14	16	19	26	35	49	78
Female	Control	1	−	−	−	−	−	−	−	−	−	−
		2	−	−	−	−	−	−	−	−	−	−
		3	±	−	−	−	−	−	−	−	−	−
		4	−	−	±	−	−	−	−	−	−	−
		5	−	−	−	−	−	−	−	−	−	−
		6	±	−	−	−	−	−	−	−	±	−
		7	−	−	−	−	−	−	−	−	−	NS
		8	−	−	−	−	−	−	−	−	−	−
	0.02% C16	9	−	−	±	−	−	−	−	−	±	NS
		10	−	−	−	−	−	−	−	−	−	−
		11	−	−	−	−	−	−	−	−	−	−
		12	−	−	−	−	−	−	−	−	−	−
		13	±	−	−	−	−	−	−	−	±	−
		14	−	−	−	−	−	−	−	−	−	NS
		15	−	−	−	−	−	−	−	−	−	−
		16	−	−	−	−	−	−	−	−	−	−
	0.2% C16	17	−	−	−	−	−	−	−	−	−	−
		18	−	−	±	−	−	−	−	−	−	−
		19	−	−	−	−	−	−	−	−	−	−
		20	−	−	−	−	−	−	−	−	±	−
		21	−	−	−	−	−	−	−	−	−	−
		22	−	−	−	−	−	−	−	−	−	−
		23	−	−	−	−	−	−	−	−	−	−
		24	−	−	−	−	−	−	−	−	±	−
	2.0% C16	25	−	−	+	+	+	Killed day 16				
		26	+	+	+	−	+	Died day 18				
		27	+	−	−	−	−	−	−	−	±	−
		28	−	−	−	+	−	−	−	Died day 28		
		29	±	+	+	+	+	Killed day 16				
		30	±	+	NS	+	+	±	Died day 19			
		31	±	+	+	+	+	−	Killed day 26			
		32	±	−	NS	Died day 13						
		33	+	+	+	+	Killed day 14					

NS = No Specimen
± = Questionable result

Table 7. *Weekly average of measured daily intake of fluid by female guinea pigs given 0.02%, 0.2% or 2.0% C16 Carrageenan in drinking water*

Group		Average weekly fluid intake (ml)									
		Weeks									
		1	2	3	4	5	6	7	8	9	10
Control	Wt.	64	57	62	63	67	59	54	56	65	64
	No.	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)
0.02% C16	Wt.	64	60	60	59	66	61	62	63	64	67
	No.	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)
0.2% C16	Wt.	60	56	57	60	68	67	62	61	63	66
	No.	(8)	(8)	(8)	(7)	(7)	(7)	(7)	(7)	(7)	(7)
2.0% C16	Wt.	58	46	63	74	72	60	76	73	73	---
	No.	(9)	(8)	(3)	(1)	(1)	(1)	(1)	(1)	(1)	(1)

Table 8. *Monthly average body weights of rats given 5% C16 Carrageenan in drinking water*

Sex	Group		Average monthly body weight (g)									
			0	1	2	3	4	5	6	7	8	9
Male	Control	Wt.	239	354	468	554	627	665	682	693	713	715
		No.	(5)	(5)	(4)	(4)	(3)	(3)	(3)	(3)	(3)	(3)
	5% C16	Wt.	237	353	459	535	559	571	581	596	582	587
		No.	(10)	(10)	(8)	(8)	(6)	(4)	(4)	(4)	(4)	(4)
Female	Control	Wt.	229	324	299	320	331	342	344	356	372	371
		No.	(5)	(5)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(3)
	5% C16	Wt.	228	258	295	325	339	351	360	376	387	384
			(10)	(10)	(8)	(8)	(6)	(6)	(6)	(6)	(5)	(4)

Table 9. *Weekly average of measured daily intake of fluid by rats given 5% C16 Carrageenan in drinking water*

Week	Average fluid intake (ml)			
	Control		5% C16	
	Male	Female	Male	Female
1.	36	36	46	40
2	44	43	52	43
3	44	43	59	48
4	51	45	58	54
5	51	42	63	59
6	52	36	67	52
7	48	36	69	48
8	41	36	69	55
9	46	31	68	45
10	45	32	66	49
11	50	37	67	50
12	48	36	69	50
13	41	35	67	52
14	50	43	68	61
15	51	38	72	61
16	58	36	72	57
17	64	32	73	55
18	55	47	74	59
19	57	51	73	63
20	55	52	75	70
21	57	46	73	68
22	64	47	73	55
23	36	37	71	46
24	55	40	73	63
25	70	56	75	74
26	72	66	75	73
27	57	52	75	75
28	54	49	75	73
29	44	45	74	73
30	39	41	73	69
31	42	40	75	74
32	36	38	75	75
33	37	39	75	72
34	40	40	75	73
35	54	43	88	74 +
36	51	42	100	77
37	49	43	97	88
38	45	45	81	69
39	49	49	92	86
40*	—	—	—	—
41	80	53	81	60
42	73	38	84	67

* Taken off C16 for 5 days during fortieth week.

\+ Increased fluid consumption consequent upon additional 25 ml provided daily.

Table 10. *Record of occult blood tests (Hematest) on feces of rats given 5% C16 Carrageenan in drinking water*

Sex	Group	Animal No.	Time (days) 8	32	77	81	88	102	109	116	123	130	137	144	188	201	231	252	266
Female	Control	31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		32	-	-	Killed 1 month														
		33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		34	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Male	Control	1	-	-	-	-	- Killed 3 months												
		2	-	-	Killed 1 month														
		3	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
		4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		5	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Female	5% C16	36	+	-	+	+	+ Killed 3 months												
		37	-	-	-	+	- Killed 3 months												
		38	-	-	+	-	+	+	-	+	+	-	+	+	+	+	+	+	+
		39	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+	+
		40	-	+	-	-	-	+	-	+	+	-	-	-	+	-	+	+	+
		41	-	+	Killed 1 month														
		42	+	-	-	-	+	+	-	+	+	-	-	+	+	+	+	+	+
		43	+	-	-	-	+	-	+	+	+	+	-	-	+	+	+	-	+
		44	+	+	Killed 1 month														
		45	+	-	-	-	+	-	-	-	+	-	-	+	+	+	+	+	+
Male	5% C16	6	+	+	+	+	+ Killed 3 months												
		7	+	+	-	+	+ Killed 3 months												
		8	+	+	Killed 1 month														
		9	-	+	-	+	+	+	-	+	+	+	-	+	+	+	+	+	+
		10	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		11	-	+	+	+	+	+	+	+	+	+ Died 4 months							
		12	-	+	+	-	-	+	-	-	+	+ Died 4 months							
		13	-	+	Killed 1 month														
		14	+	+	-	-	+	+	-	-	+	+	+	+	+	+	+	+	+
		15	-	+	-	+	+	+	-	-	+	-	+	+	+	+	+	+	+

Table 11. *Hematological data on rats given 5% C16 in drinking water for 7 months*

No.	Sex	RBC ($X10^6$)	Hemoglobin (g%)	PCV (%)	Leucocytes WBC ($X10^3$)	Differential N	L	M	E
			Controls at 2 wk						
1	Male	6.83	15.3	39.5	20.7	2	98		
3	Male	7.42	13.3	40.0	11.7	1	98	1	
32	Female	5.72	14.3	39.5	13.1	1	98	1	
33	Female	6.30	14.2	36.5	9.3		99		1
34	Female	5.64	13.9	37.0	15.4	2	97	1	
35	Female	6.71	16.2	38.0	7.1	2	97	1	
			C16 rats at 2 wk						
7	Male	6.59	13.9	41.5	14.2		99	1	
10	Male	6.47	14.7	37.5	15.3		99	1	
11	Male	6.15	14.7	38.0	16.6		100		
12	Male	6.94	14.3	38.0	16.7	1	98	1	
13	Male	6.48	14.2	38.0	16.5	7	91	1	1
36	Female	5.47	15.8	37.5	13.8	1	99		
37	Female	6.64	16.8	38.5	11.6	6	94		
38	Female	6.50	16.0	40.5	12.2	4	94	1	1
40	Female	5.80	15.8	39.5	10.5	2	96	2	
41	Female	6.08	15.3	38.5	12.7		100		
			Controls at 7 months						
3	Male	8.41	16.4	53.5					
4	Male	6.25	14.2	45					
5	Male	8.58	17.9	59					
33	Female	6.91	16.0	49					
34	Female	7.16	16.0	49.5					
35	Female	9.21	18.1	52					
			C16 rats at 7 months						
9	Male	8.95	17.6	56					
10	Male	8.24	16.9	54					
14	Male	7.59	16.2	52.5					
15	Male	7.77	17.2	55.5					
38	Female	7.49	15.2	50.5					
39	Female	8.30	17.4						
40	Female	7.46	18.1	53.5					
43	Female	6.64	14.6	45					
45	Female	6.80	14.2	47.5					

Table 12. *Individual bimonthly body weights (kg) of monkeys given 2% C16 in drinking water*

Sex	Group	Animal No.	Time (wk) 0	4	6	8	10
Female	Control	1028	2.94	-	2.94	3.38	3.50
Male	Control	1010	2.98	-	2.98	3.45	3.70
Female	2% C16	1021	2.84	2.91	2.96	2.67	2.40
		1022	2.76	2.81	2.77	2.80	2.61
		1024	2.85	2.62	2.50	Killed 7 wks Final body wt. 2.46	
Male	2% C16	1006	3.06	3.00	2.89	2.78	2.65
		1009	2.81	2.94	3.10	2.92	2.97
		1017	2.85	2.99	3.11	2.75	2.41
							Killed 10 wks Final body wt. 2.14

Table 13. *Individual bimonthly body weights of monkeys during recovery period after consumption of C16*

Sex	Group	Animal No.	Time (wk) 0	2	4	6	8	10	12	14	16	19	21	23
Female	Control	1028	3.50	3.45	3.56	3.46	3.87	4.02	3.83	4.10	4.03	4.13	Transferred	
Male	Control	1010	3.70	3.35.	3.43	3.32	3.54	3.53	3.49	3.60	3.66	3.81	To Stock	
Female	2% C16	1021		2.58	2.42	2.64	2.77	2.67	2.41	2.62	2.45	2.22	Killed 20 wks	
		1022		2.52	2.49	2.60	2.43	2.41	2.20	2.63	2.78	--	2.96	Killed 24
Male	2% C16	1006		2.82	3.19	3.20	3.42	3.44	3.37	3.50	3.54	3.68	3.70	Killed 24
		1009		2.80	3.05	3.06	3.61	3.62	3.45	3.80	3.62	3.74	3.86	Killed 24

Table 14. *Individual bimonthly body weights (kg) of monkeys given C16 in drinking water*

Sex	Group	Animal No.	Time (wk) -7	-3	-1	1	3	5	7	9	11	13	
Female	Control	1028	2.94	-	2.94	3.38	3.50	3.45	3.50	3.46	3.87	4.02	
Male	Control	1010	2.98	-	2.98	3.45	3.70	3.35	3.43	3.32	3.54	3.53	
Female	1% C16	1020	2.99	3.14	3.27	3.11	3.17	3.16	3.10	2.96	3.34	3.27	Killed 14 wk
Male	1% C16	1005	2.60	2.67	2.86	2.67	2.80	2.58	2.48	2.44	2.81	2.80	Killed 14 wk
Female	0.5% C16	1023	2.41	2.75	2.85	2.72	2.82	2.77	2.77	2.73	3.16	2.75	Killed 14 wk
Male	0.5% C16	1008	2.99	2.95	3.07	2.99	3.09	3.03	3.11	3.00	3.19	3.22	Killed 14 wk

Table 15. Record of occult blood tests (Hematest) on feces of monkeys given 2% C16 in drinking water

Sex	Group	Animal No.	Time (day) 6	19	20	26	29	40	48	50	55	62	68	70	72	
Female	Control	1028	-	-	-	-	-	-	-	-	-	-	-	-	-	
Male	Control	1010	-	+	-	-	-	-	-	-	-	-	-	-	-	
Female	2% C16	1021	-	+	-	+	+	-	+	+	-	+	+	-	+	
		1022	+	+	-	+	+	-	+	+	+	+	+	+	+	
		1024	-	-	-	-	+	-	+	Killed day 49						
Male	2% C16	1006	-	+	-	+	+	+	+	-	+	+	+	+	+	
		1009	-	+	+	+	-	-	+	+	+	+	+	+	+	
		1017	-	+	-	+	+	-	+	+	+	-	+	+	+	Killed day 76

Table 16. *Record of occult blood tests in feces of monkeys during recovery period following ingestion of 2% C16 in drinking water.*

Sex	Group	Animal No.	Time (day) 75	77	79	82	84	86	89	91	96	98	103	105	107	110	112	114	117	121	124	126
Female	Control	1028	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Male	Control	1010	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Female	2% C16	1021	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+
		1022	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Male	2% C16	1006	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-
		1009	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+

Sex	Group	Animal No.	Time (day) 131	133	135	138	140	142	146	147	148	149	152	154	159	161	166	168	170	173	180	198
Female	Control	1028	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-
Male	Control	1010	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Female	2% C16	1021	+	+	+	-	-	-	-	-	-	-	+	-	-	+	+	+	+	-	-	-
		1022	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	-
Male	2% C16	1006	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	-	-	-
		1009	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 17. *Record of tests for occult blood in the feces of monkeys given 1.0% and 0.5% C16 in drinking water*

Sex	Group	Animal No.	Time (day) 1	2	7	14	20	22	24	27	29	31	34	36	38	41	43	48	50	55	57	59
Female	Control	1028	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-
Male	Control	1010	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Female	1% C16	1020	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Male	1% C16	1005	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Female	0.5% C16	1023	-	-	-	-	+	+	-	+	+	+	-	+	+	-	-	+	+	+	+	+
Male	0.5% C16	1008	-	-	+	+	-	-	+	+	+	+	+	-	-	-	+	-	+	-	+	+

Sex	Group	Animal No.	Time (day) 62	64	66	69	73	76	78	83	85	87	90	92	94	98	
Female	Control	1028	-	-	-	-	-	-	-	-	-	-	-	-	-	+	
Male	Control	1010	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Female	1% C16	1020	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Killed day 98
Male	1% C16	1005	+	-	-	-	+	+	+	+	+	+	+	+			Killed day 92
Female	0.5% C16	1023	-	-	+	+	-	+	+	+	+	+	+	+	+	+	Killed day 98
Male	0.5% C16	1008	+	-	-	-	-	+	+	+	+	+	+				Killed day 92

Table 18. *Weekly average of measured daily intake of fluid by monkeys given Cl6 in drinking water*

Week	Ave. fluid intake (ml)*			
	Original groups		New groups	
	Controls	2% Cl6	1% Cl6	0.5% Cl6
1	398	396		
2	396	388		
3	398	383		
4	397	345		
5	398	260		
6	400	320		
7	398	265	372	365
8	400	356	400	400
9	400	310	400	400
10	400	288	398	400
11	400	246	386	390
12	400	255	365	370
13			400	400
14			400	400
15			400	400
16			400	400
17			400	400
18			400	400
19			400	400
20			400	400
21			400	400
22			400	400
23			400	400
24			400	400

* 400 ml represents the total volume of the water bottle; the amount spilled cannot be ascertained.

Table 19. *Hematological data on monkeys before and after administration of C16 in drinking water and following a recovery period*

Monkey No.	Sex	Level of C16 (%)	Duration (wk) C16	Duration (wk) Recovery	RBC ($X10^6$)	Hemoglobin (%)	PCV (%)	Leucocytes WBC ($X10^3$)	Leucocytes Differential N	L	M	E	B
1023	Female	0.5	0	--	6.77	14.5	40.5	9.3	23	76	1		
			9	--	4.35	14.5	36.5	5.8	18	76	4	2	
1020	Female	1.0	0	--	7.19	14.7	45.5	14.4	20	79	1		
			9	--	4.98	12.9	36.0	8.7	10	88	2		
1021	Female	2.0	0	--	7.02	15.0	41.0	14.7	32	68			
			9	--	4.75	12.4	34.0	11.4	42	56	2		
			9	15	6.18	14.1	44.0	7.7	44	51	3	2	
1022	Female	2.0	0	--	8.19	16.5	49.5	19.1	32	66	2		
			9	--	5.43	16.8	41.5	12.6	30	67	3		
			9	24	7.08	17.6	53.5	20.7	31	61	3	2	3
1024	Female	2.0	0	--	6.89	16.2	44.5	22.6	23	74	3		
			7	--	6.27	16.0	43.5	15.6	13	83	4		
1008	Male	0.5	0	--	6.40	15.3	41.5	16.6	26	74			
			9	--	4.51	14.3	37.5	10.8	29	66	4	1	
1005	Male	1.0	0	--	6.71	13.3	35.5	9.9	3	95	2		
			9	--	4.77	11.0	34.5	19.1	7	90	1	2	
1006	Male	2.0	0	--	6.15	13.9	39.5	7.5	11	85	4		
			9	--	4.91	12.7	35.0	13.4	20	76	4		
			9	24	6.52	14.9	46.0	36.9	38	61		1	
1009	Male	2.0	0	--	6.42	16.9	40.5	13.7	25	72		3	
			9	--	5.35	14.7	40.0	11.9	22	77	1		
			9	24	5.61	15.0	46.5	16.7	10	84	3	3	
1017	Male	2.0	0	--	6.04	13.1	37.5	13.1	20	80			
			9	--	4.62	13.3	34.5	9.5	4	95		1	

Table 20. *Weekly average body weights (g) of guinea pigs given 1% HMR in drinking water*

Sex	Group		Time (wk) 0	1	2	3	4	5	6	7	8	9	10	11
Female	Control	Wt.	664	623	684	710	711	718	746	725	736	737	752	759
		No.	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
	1% HMR	Wt.	597	629	679	723	704	699	732	748	747	808	760	799
		No.	(10)	(10)	(10)	(10)	(10)	(9)	(8)	(7)	(7)	(4)	(4)	(3)
Male	Control	Wt.	582	599	711	760	755	759	814	844	881	889	903	904
		No.	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
	1% HMR	Wt.	714	698	737	778	750	752	762	793	792	843	857	858
		No.	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(3)	(3)	(3)

			Time (wk) 12	13	14	15	16	17	18	19	20	21	22	23	24
Female	Control	Wt.	746	721	722	725	735	751	781	786	793	802	846	811	825
		No.	(5)	(5)	(4)	(4)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
	1% HMR	Wt.	783	782	712	647	660	663	725	728	725	729	712	740	645
		No.	(3)	(3)	(3)	(3)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)
Male	Control	Wt.	905	906	856	808	794	823	847	851	1032	1059	1071	1160	1221
		No.	(4)	(4)	(4)	(4)	(4)	(3)	(3)	(3)	(2)	(2)	(2)	(2)	(2)
	1% HMR	Wt.	857	857	795	683	756	754	775	787	820	847	866	885	890
		No.	(3)	(3)	(3)	(3)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)

Table 21. Weekly average of measured daily uptake of fluid (ml) by guinea pigs given 1% HMR in drinking water

Sex		Time (wk) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Male		68	64	72	68	64	70	75	65	65	63	73	62	64	68	73	72	75	75	74	75	73	75	72	75
	No.	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
Female		55	72	68	68	52	58	71	71	41	68	56	72	68	56	72	71	75	75	76	75	75	73	75	75
	No.	(10)	(10)	(10)	(10)	(9)	(8)	(7)	(7)	(4)	(4)	(3)	(3)	(3)	(3)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)

Table 22. *Monthly average body weights (g) of rats given 1% HMR in drinking water*

Sex	Group		Time (months) 0	1	2	3	4	5	6
Male	Control	Wt.	260	408	506	543	560	590	582
		No.	(5)	(5)	(5)	(5)	(5)	(5)	(4)
	1% HMR	Wt.	260	402	518	554	573	592	627
		No.	(10)	(10)	(10)	(10)	(10)	(8)	(7)
Female	Control	Wt.	183	246	293	317	327	345	363
		No.	(5)	(5)	(5)	(5)	(5)	(5)	(4)
	1% HMR	Wt.	183	235	283	298	314	333	339
		No.	(10)	(10)	(10)	(10)	(10)	(9)	(8)

Table 23. Weekly average of measured daily intake of fluid by rats given 1% HMR in drinking water

	Average fluid intake (ml)			
	Control		1% HMR	
	Male	Female	Male	Female
1	48	42	35	33
2	51	46	39	31
3	46	41	38	37
4	42	36	39	38
5	46	42	41	36
6	43	42	38	43
7	42	39	40	39
8	47	41	38	35
9	46	42	41	38
10	45	42	40	35
11	49	41	41	38
12	51	48	48	42
13	56	51	50	53
14	60	50	62	60
15	55	42	53	41
16	50	43	43	43
17	43	41	49	44
18	42	44	46	41
19	40	39	41	35
20*	36	34	39	29
21	35	34	41	32
22	38	34	36	33
23	36	34	39	29
24	34	31	35	29
25	36	29	35	30
26	36	33	35	31

* Slight lower figures after week 20 reflect use of a new watering device with less waste.

Table 24. *Hematological data on rats given 1% HMR in drinking water for 4 months*

No.	Sex	RBC ($X10^6$)	Hemoglobin (g%)	PCV (%)	Differential N	E	L	M	B
			Controls						
1	Male	7.16	17.6	55	22	2	74	2	
2	Male	6.12	17.4	51.5	19	2	76	3	
3	Male	8.37	16.4	50	9	2	88	1	
4	Male	8.07	16.2	49.5	13	1	86		
5	Male	7.99	16.4	51	16		82	2	
16	Female	6.79	18.6	59	14	2	82	1	1
17	Female	6.35	17.6	50	8	1	90	1	
18	Female	7.90	19.2	57	15		84	1	
19	Female	6.08	18.1	51.5	12	1	87		
20	Female	6.48	17.6	50.5	21	2	76	1	
			1% HMR						
6	Male	7.10	14.6	45.5	15	7	76	2	
7	Male	7.93	16.7	51	16		84		
8	Male	7.04	15.4	47.5	21	2	76	1	
10	Male	9.07	19.2	56.5	12	1	86	1	
11	Male	7.07	16.9	48.0	16	1	83		
21	Female	6.27	16.2	45.5	16	2	81	1	
22	Female	6.03	16.7	47.5	15	84	1		
23	Female	6.00	17.2	46.5	26	1	71	2	
24	Female	7.33	18.9	59	18	1	81		
25	Female	6.42	17.8	52	8	91	1		

Table 25. *Daily average food consumption (g) by male gerbils given 5% C16 or 5% HMR in the diet*

Group	Time (wk) 1	2	3	4	5	6	7	8	9	10	11	12
Control	5.0	5.2	5.2	5.0	4.7	4.6	4.7	4.4	4.2	4.6	4.3	4.6
5% C16	4.9	5.1	5.6	5.9	5.4	5.2	5.1	5.2	4.4	4.7	4.8	4.4
5% HMR	5.8	5.9	5.8	5.6	5.4	5.4	4.9	5.3	5.1	5.2	5.1	5.1

	Time (wk) 13	14	15	16	17	18	19	20	21	22	23
Control	5.5	3.1	3.4	3.4	3.2	3.7	3.6	3.3	3.5	3.0	3.8
5% C16	5.4	3.8	3.6	3.5	3.6	3.6	3.7	3.4	3.6	2.9	4.0
5% HMR	5.6	4.7	4.7	4.0	4.1	4.2	3.8	4.0	4.0	3.5	3.8

Table 26. *Weekly average body weight (g) of male gerbils given 5% C16 and 5% HMR in the diet*

Group		Time (wk) 0	1	2	3	4	5	6	7	8	9	10	11
Control	Wt.	69	74	78	82	83	83	84	86	86	87	92	93
	No.	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
5% C16	Wt.	69	70	77	80	82	88	86	88	89	93	96	97
	No.	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
5% HMR	Wt.	69	70	77	79	80	86	86	88	87	90	95	96
	No.	(5)	(5)	(5)	(5)	(5)	(4)	(4)	(4)	(4)	(4)	(4)	(4)

		Time (wk) 12	13	14	15	16	17	18	19	20	21	22	23	24
Control	Wt.	92	89	91	91	92	91	92	90	90	90	91	88	88
	No.	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
5% C16	Wt.	99	98	100	100	101	100	102	101	98	100	100	98	96
	No.	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
5% HMR	Wt.	96	95	97	99	100	99	101	99	98	100	100	98	94
	No.	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)

Table 27. *Weekly average of measured daily intake of fluid by monkeys given 1% HMR in drinking water*

Week	Ave. daily intake of fluid (ml)*	
	Control	1% HMR
1	398	365
2	396	396
3	398	386
4	397	376
5	398	360
6	400	280
7	398	375
8	400	388
9	400	386
10	400	392
11	400	376
12	400	336

* 400 ml represents the total volume of the water bottle; the amount spilled cannot be ascertained.

Table 28. *Individual weekly body weights of monkeys given 1% HMR in drinking water*

Sex	Group	Monkey No.	Time (wk) 0	4	6	8	10	12	14	16	18
Female	Control	1029	3.14	-	3.14	3.17	3.42	3.16	3.21	3.17	3.56
Male	Control	1016	2.84	-	2.84	2.97	2.87	2.85	2.88	2.91	2.88
Female	1% HMR	1026	2.68	2.91	2.90	Killed 7 wk	Final body wt. 2.87				
		1034	3.08	3.45	3.36	3.41	3.26	3.30	3.28	3.21	3.47
		1035	2.92	3.28	3.27	3.09	3.30	3.16	3.23	3.05	3.22
Male	1% HMR	1002	2.77	3.15	3.15	3.16	3.20	Killed 11 wk	Final body wt. 3.23		
		1003	2.93	3.43	3.34	3.19	3.39	3.20	3.32	3.24	3.63
		1004	2.91	3.24	3.26	3.24	3.44	3.38	3.27	3.20	3.66

Table 29. *Individual body weights of monkeys in dose-ranging experiment with HMR**

Sex	Group	Monkey No.	Time (wk) 0	2	4	6	8	10	12	
Female	Control	1029	3.58	3.83	4.10	4.03	4.04	4.13	4.21	Killed 12 wk
Female	HMR	1034	3.52	3.43	3.65	3.65	3.72	3.96	3.98	Killed 12 wk
		1035	3.45	3.48	3.55	3.50	3.51	3.54	3.61	Killed 12 wk
Male	HMR	1003	3.63	3.50	3.57	3.58	3.68	3.98	4.06	Killed 12 wk
		1004	3.73	3.65	3.90	3.98	4.18	4.27	4.31	Killed 12 wk

* Dose-range –
3 days – 50 mg/kg
150 mg/kg
5 days – 300 mg/kg
7 days – 600 mg/kg
900 mg/kg
1200 mg/kg
54 days – 1250 mg/kg

Table 30. *Record of occult blood tests (Hematest) in monkeys given 1% HMR in drinking water*

Sex	Group	Monkey No.	Time (day) 6	19	20	26	29	40	48	50	55	62	68	70	72*	75	77	79	82	84	86	89
Female	Control	1029	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–
Male	Control	1016	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	+
Female	1% HMR	1026	–	–	–	–	–	–	–	Killed day 49												
		1034	–	–	–	–	–	–	–	–	–	–	+	+	+	–	–	–	–	–	–	–
		1035	–	–	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–	–	–
Male	1% HMR	1002	–	–	–	–	–	–	–	–	–	+	–	–	–	–	Killed day 76					
		1003	–	–	–	–	–	–	–	–	–	–	+	–	–	+	–	–	–	–	–	–
		1004	–	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–

Sex	Group	Monkey No.	Time (day) 96	98	103	105	107	110	112	114	117	121	124	126	131	133	135	138	140	142	146
Female	Control	1029	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Male	Control	1016	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	+	–	–	–
Female	1% HMR	1034	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	+	–	+
		1035	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	+
Male	1% HMR	1003	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–
		1004	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–

* Day on which administration of HMR ceased, i.e. start of recovery period.

Table 31. Record of occult blood tests (Hematest) in monkeys on dose-ranging study with HMR

Sex	Group	Animal No.	Time (day) 147*	148	149	150	152	153	154	155	156	157	159	160	161	162	163	164	166	167	168	169	170	171
Female	Control	1029	-	-	-	-	-	+	-	+	+	-	-	-	NS	-	-	Menses		+	-	-	-	-
Female	HMR	1034	Menses	-	-	-	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-
		1035	Menses	-	-	-	-	-	+	-	+	-	-	-	+	+	-	-	-	-	-	-	+	-
Male	HMR	1003	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NS	-	-	-	+	-
		1004	-	-	-	-	-	+	-	-	-	+	-	-	NS	-	-	-	-	-	-	-	-	-

Sex	Group	Animal No.	Time (day) 173	174	175	176	177	180	181	182	183	184	187	188	189	190	191	192	194	195	196	197	198	201
Female	Control	1029	-	-	-	-	-	-	-	-	-	-	-	-	-	Menses		-	-	-	-	-	-	-
Female	HMR	1034	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
		1035	-	+	-	+	-	-	-	+	-	+	-	NS	-	+	-	NS	+	-	+	-	+	-
Male	HMR	1003	-	+	+	-	-	-	-	-	-	-	-	-	-	-	NS	-	-	-	-	-	-	+
		1004	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	NS	+	-	-	NS	+	-

Sex	Group	Animal No.	Time (day) 202	203	204	205	208	209	210	211	212	215	216	217	218	219	222	223	224	225	226	229	230	231	232
Female	Control	1029	-	-	-	-	-	-	-	-	-	-	-	Menses		-	-	-	-	-	-	-	-	-	-
Female	HMR	1034	-	-	-	-	-	-	-	-	-	-	-	-	Menses	+	-	-	-	-	-	-	-	-	-
		1035	+	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Male	HMR	1003	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		1004	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* Time from start of original administration of HMR in drinking water.
NS - No Specimen

Table 32. *Hematological data on control and test monkeys*

Monkey No.	Sex	Before HMR	After HMR	RBC ($X10^6$)	Hemoglobin (g%)	PCV (%)	Leucocytes WBC ($X10^3$)	Leucocytes Differential N	L	M	E	B
				Monkeys on 1% HMR for 9 wk								
1002	Male	+		6.69	13.5	37.5	12.3	18	79	3		
			+	5.14	15.0	37.5	9.4	31	67	2		
1003	Male	+		6.03	16.5	39.5	12.6	14	86			
			+	4.35	12.6	34.0	21.7	32	67	1		
1004	Male	+		5.85	14.3	35.5	28.7	18	82			
			+	4.06	12.0	28.0	15.6	28	69	1	2	
1026	Female	+		6.67	15.0	40.0	15.2	26	73	1		
			+	5.42	12.7	37.0	10.4	27	73			
1034	Female	+		7.12	15.3	40.5	18.7	10	89	1		
			+	4.00	12.2	36.5	12.5	39	60	1		
1035	Female	+		6.01	12.7	32.0	19.4	70	30			
			+	4.48	12.6	32.5	16.5	25	70	2	3	
				Control monkeys on plain water for 22 wk								
1028	Female			5.37	13.1	41.0	13.8	27	68	1	4	
1029	Female			4.92	12.3	39.0	11.2	43	51	4	1	1
1010	Male			5.36	14.6	44.5	6.6	23	73	2	1	1
1016	Male			4.45	12.1	38.0	6.7	12	74	5	6	3
				Monkeys on dose-ranging study with HMR for 12 wk								
1034	Female			5.40	14.2	42.0	13.1	8	89	2	1	
1035	Female			4.92	13.7	37.5	16.3	36	61		3	
1003	Male			5.53	15.4	45.0	9.6	11	87		2	
1004	Male			5.52	14.2	42.0	15.0	8	78	2	11	1

Table 33. *Comparison of histopathological findings in monkeys given C16 carrageenan in drinking water*

Changes observed	Level of C16 (%)...	2%		1%	0.5%
	Duration (wk)...	7	11	14	14
	No. of animals examined...	1	1	2	2
Inflammatory changes					
Capillary congestion: cecum, colon		+	+	+	+
Mucosal edema: cecum		-	+	-	-
colon		-	+	+	+
Cellular infiltration - cecum: M*		-	+	-	-
L		+	+	+	-
P		+	+	+	-
colon: M*		+	+	+	+
L		+	+	+	+
P		+	+	+	+
Damage to epithelium					
Cecum - erosion of epithelium		-	+	-	-
ulceration: focal		-	+	-	-
: penetrating†			+	-	-
Colon - erosion of epithelium		-	+	+	+
ulceration: focal		-	+	-	-
: penetrating†			+	-	-
Crypt abscesses					
Sites - cecum		-	+	-	-
colon		-	3+	2+	+
Character - necrosis of crypt ep.: cecum		-	-	-	-
colon		-	+	+	+
decreased mucus inadjacent glands		-	-	-	-
Ep. necrosis - throughout crypt: cecum		-	-	-	-
colon		+	+	+	-
- in depths of crypt: cecum		-	-	-	-
colon		-	-	-	-
Epithelial hyperplasia		-	-	-	-

*M - Macrophages (Further ultrastructural study of the nature of these cells is under way); L - Lymphocytes; P - Plasma cells

† Penetrating to, and involving the muscularis mucosae

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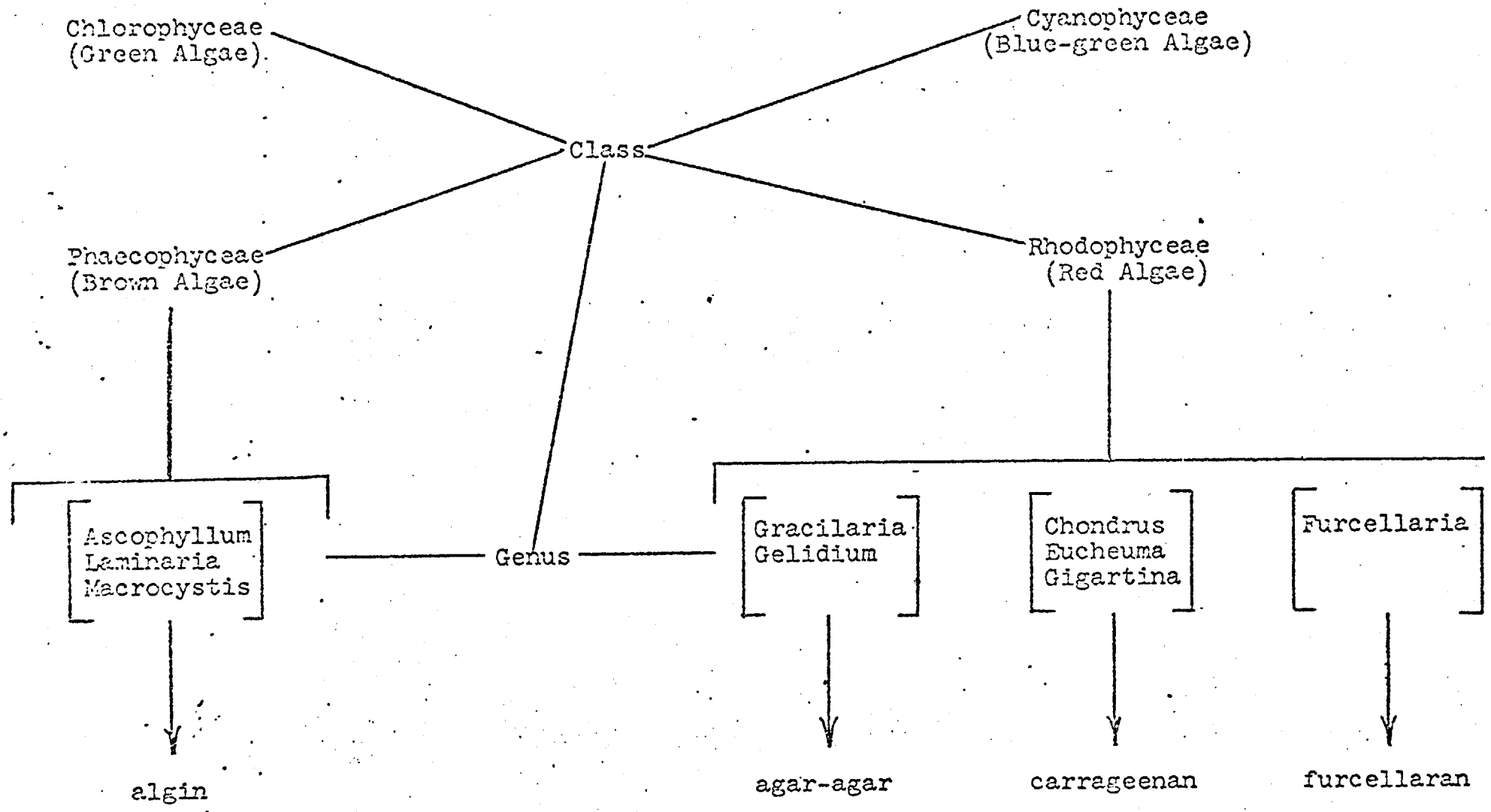
TECHNICAL SEMINAR

Marine Colloids, Inc.
Springfield, N.J.

MARINE ALGAE CLASSIFICATION



2

Excerpted from U. S. Federal Register, Friday, October 6, 1961

121.1066 Carrageenan

The food additive carrageenan may be safely used in food in accordance with the following prescribed conditions:

(a) The food additive is the refined hydrocolloid prepared by aqueous extraction from the following members of the families Gigartinaceae and Solieriaceae of the class Rhodophyceae (red seaweed):

Chondrus crispus
Chondrus ocellatus
Eucheuma cottonii
Eucheuma spinosum
Gigartina pistillata
Gigartina radula
Gigartina stellata

(b) The food additive conforms to the following conditions:

1. It is a sulfated polysaccharide, the dominant hexose units of which are galactose and anhydrogalactose.

2. Range of sulfate content: 20 percent to 40 percent on a dry-weight basis.

CARRAGEENAN*

(Irish Moss Extract, Carrageenin, Carragheenin, Chondrus Extract)

YEAR	WORKERS	RESULT
?		Blancmange
1860-1890	Bente, Haedicke, Hass	Presence of d-galactose
1921	Haas	Ester Sulfate
1921	Haas, Russell-Wells	Two Fractions (?)
ca 1935		Commercial Production
1953	Smith & Cook	Separation of Two Fractions (Kappa & Lambda)
1955	O'Neill	Chemical Structure of Two Fractions (Kappa & Lambda)
1963-1967	Rees	Further Characterization of Kappa & Lambda Third Fraction (Iota)

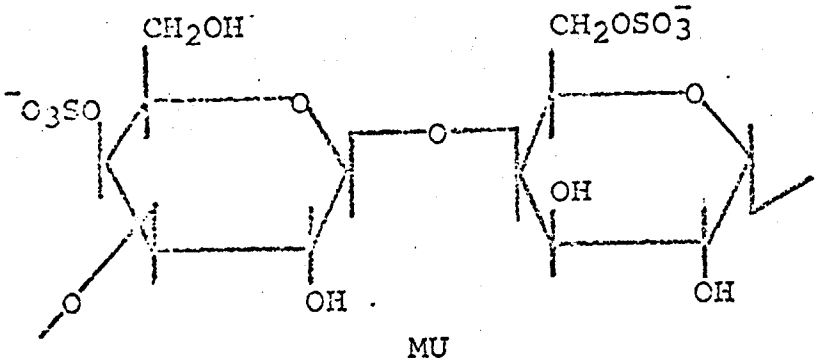
*Revised spelling passed by the Organic Chemistry Division of the American Chemical Society in 1959.

COMMON STRUCTURAL FEATURES OF RED SEAWEED POLYGALACTANS

——— G 1β4 G 1α3 G 1β4 G 1α3 G ———

POLYGALACTAN	FRACTION	SUGAR UNITS
Agar Agar	Agarose	D-galactose 3,6-anhydro-L-galactose
	Agaropectin	
Carrageenan	Lambda	D-galactose-2-sulfate D-galactose-2,6-disulfate
	Kappa	D-galactose-4-sulfate 3,6-anhydro-D-galactose
	Iota	D-galactose-4-sulfate 3,6-anhydro-D-galactose-2-sulfate
Furcellaran		D-galactose D-galactose-4-sulfate 3,6-anhydro-D-galactose

CARRAGEENAN STRUCTURES



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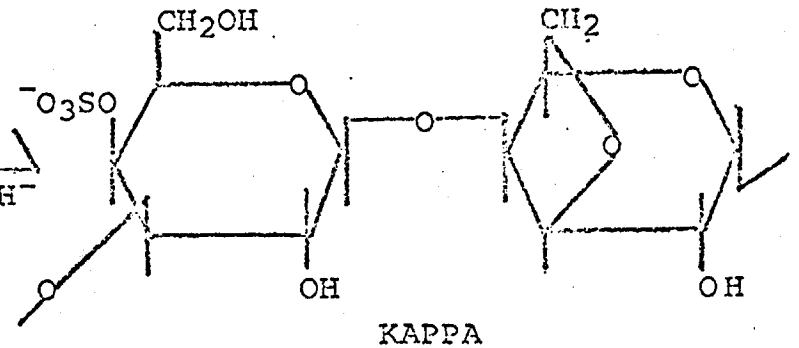
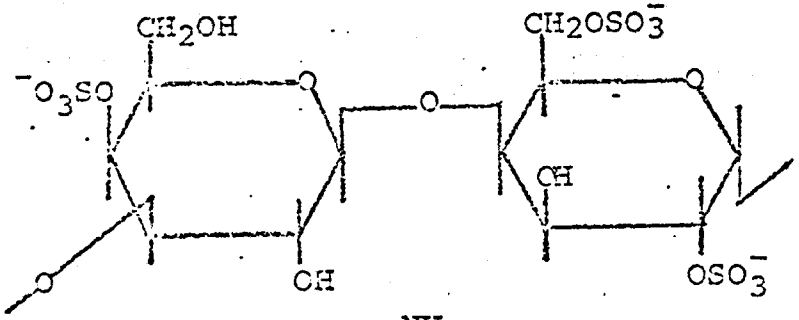


KAPPA



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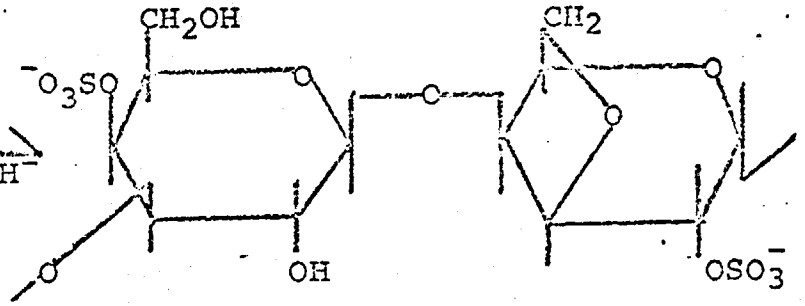

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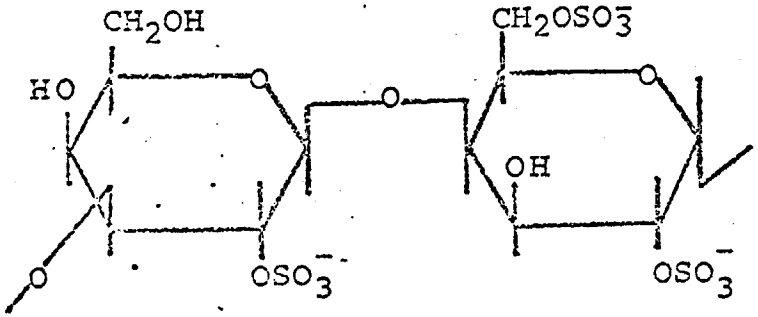
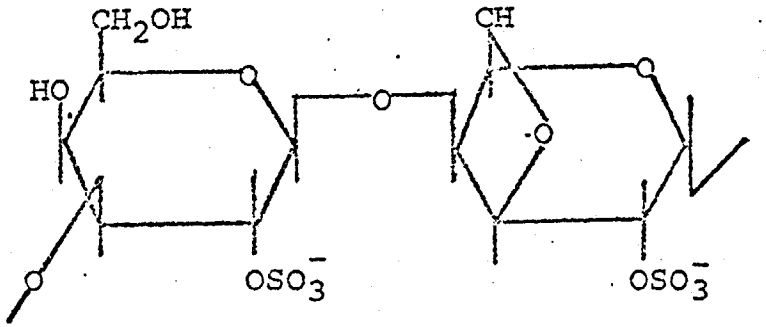


LAMBDA



Alkali Treated
LAMBDA

COMPARISON OF KAPPA, LAMBDA and IOTA CARRAGEENAN

	KAPPA	LAMBDA	IOTA
Cold Water Solubility	From limited to very high swelling. Sodium salt produces free flowing solutions	All salts fully soluble, solutions are free flowing	Thixotropic dispersions with calcium salt
Effect of cations	Gels most strongly with potassium	Non-gelling	Gels most strongly with calcium
Type of gel	Brittle with syneresis, reversible with heat	Non-gelling	Elastic with no syneresis Reversible with heat
Solubility in concentrated sugar solutions	Soluble hot	Soluble hot	Difficultly soluble
Solubility in concentrated solutions of various salts	Insoluble cold and hot	Insoluble cold and hot	Soluble hot
Solubility in cold milk	Practically insoluble	Dispersible with thickening or gelling	Practically insoluble
(with added $Na_4P_2O_7$)	(Thickens or gels)	(Increased thickening or gelling)	(Thickens or gels)

GENERAL PROPERTIES OF κ, λ, ι -CARRAGEENAN

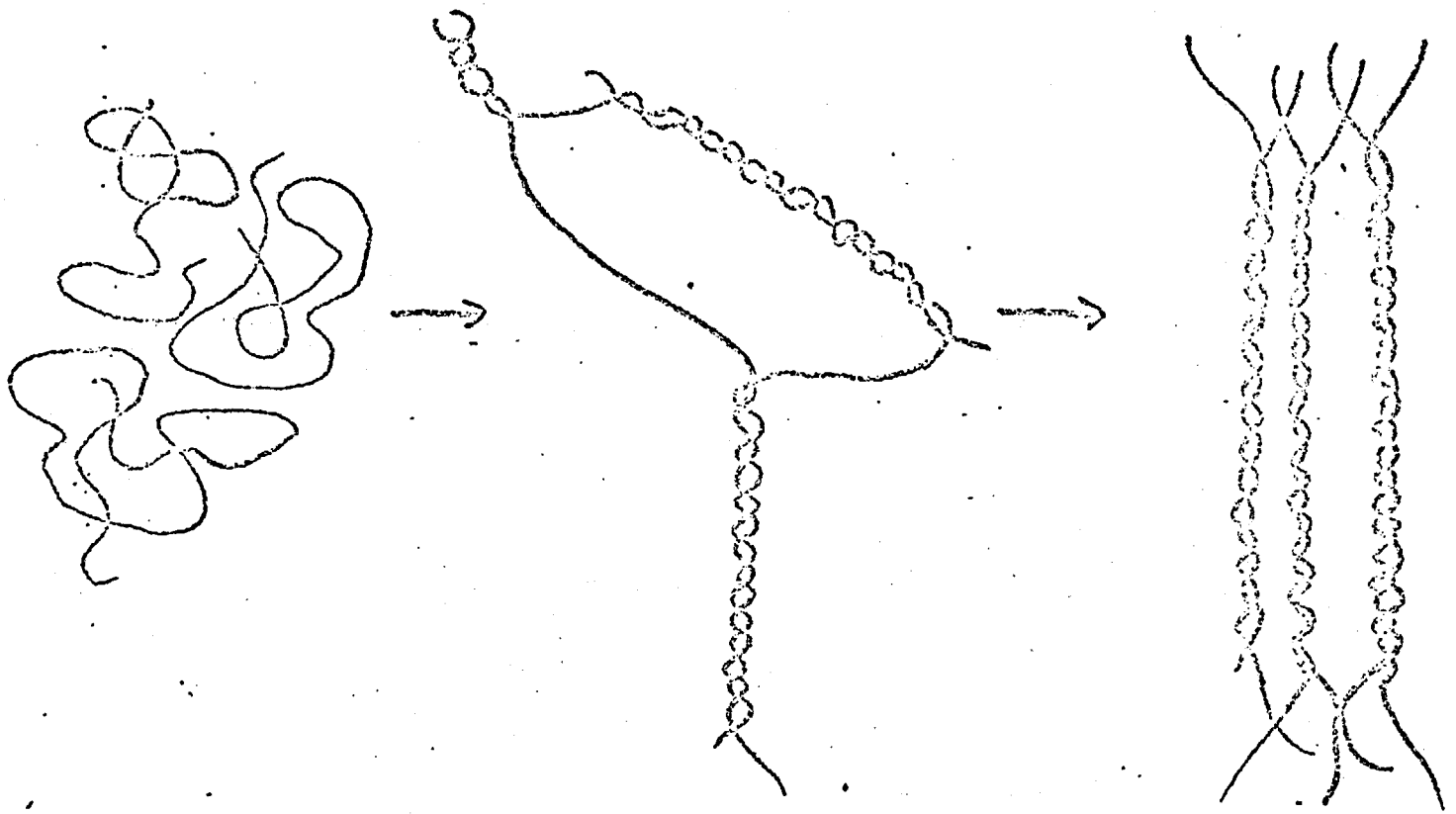
Soluble in water - may require heat

Insoluble in organic solvents

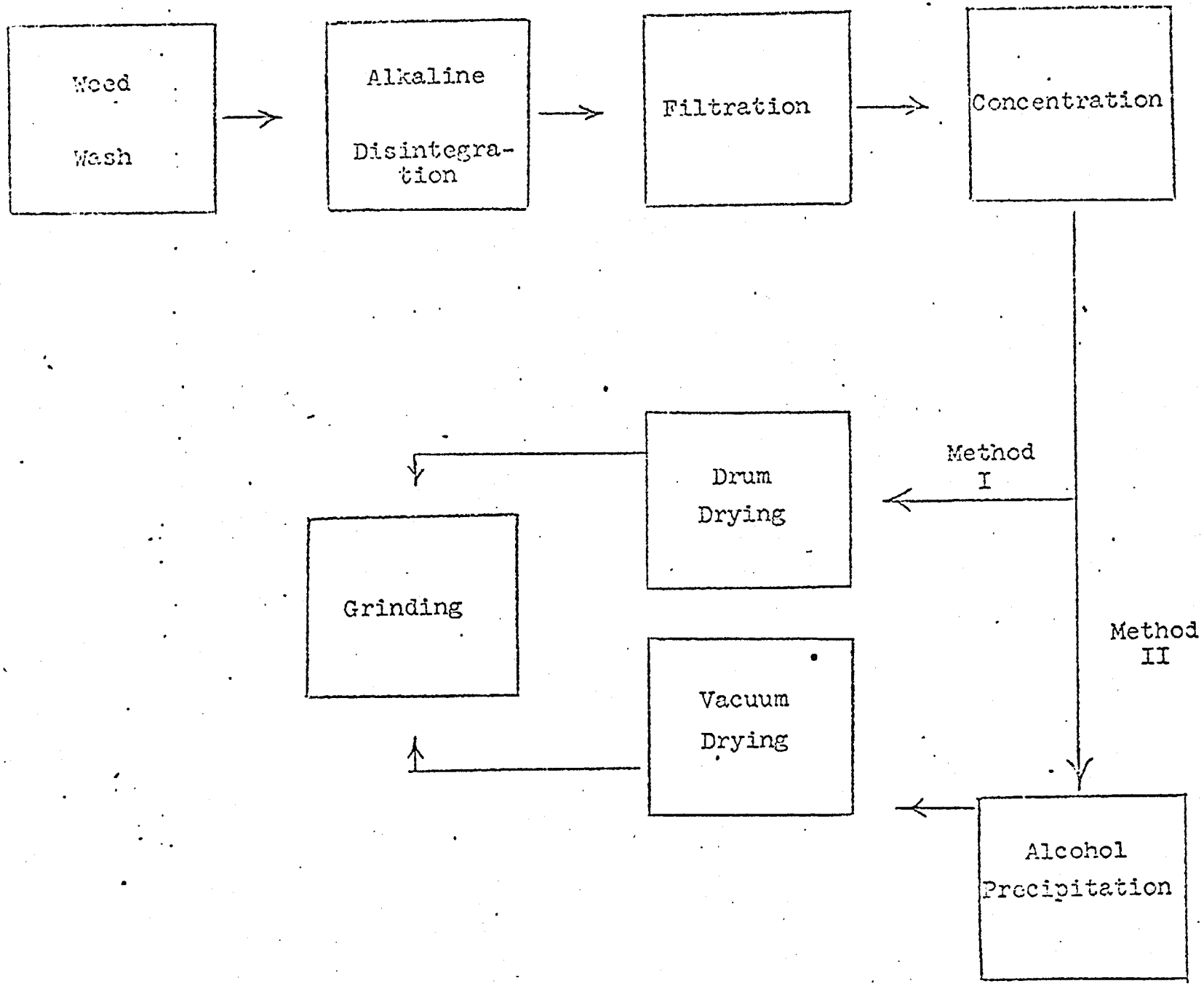
Water miscible solvents, e.g., alcohol, propylene glycol, glycerine - may be incorporated into carrageenan solution. Concentration of solvent tolerated depends on molecular weight of carrageenan, type of carrageenan and balance of cations, method of incorporation of solvent.

Compatibility - Generally compatible with anionic and non-ionic products. Incompatible with cationics, e.g., detergents, quaternaries, proteins below and near isoelectric point, certain amines (low molecular weight amines, e.g., TEA, depress viscosity).

MECHANISM FOR GELATION BY κ AND λ- CARRAGEENANS



COMMERCIAL PRODUCTION OF CARRAGEENAN



USE OF CARRAGEENAN IN COSMETICS AND PHARMACEUTICALS

APPLICATION	FUNCTION
Lotions and creams	Bodying, slip, rub-out
Hydroalcoholic lotion and creams	Bodying, emolliency, rub-out
Shampoos	Foam stabilization, thickening, gelling
Toothpaste	Bodying, foam stabilization
Ulcer products	Protein reactivity
Cough preparations	Coating
Salves	Bodying
Chewable tablets	Reduce chalkiness
Medicinals (milk magnesia)	Suspension of insoluble ingredients
Laxatives (liquids)	Oil in water emulsion stabilization

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TYPICAL CARRAGEENAN PRODUCTS MANUFACTURED BY

MARINE COLLOIDS, INC.

ALCOHOL PRECIPITATED EXTRACTS

For Use in Water Systems

Gelcarin L WG (low water gel kappa type)

Gelcarin GH (high water gel kappa type)

SeaGel GH (high water gel kappa type)

Gelcarin SI (medium water gel iota type)

Viscarin (non-gelling sodium form of kappa/lambda mixture)

Viscarin 402 (non-gelling lambda type)

For Use in Milk Systems

Gelcarin L MR (low milk gel kappa type)

Gelcarin M MR (medium milk gel kappa type)

Gelcarin H MR (high milk gel kappa type)

Gelcarin MAC (medium milk gel iota type)

Gelloid C, D, DC (high milk reactive kappa types with sugar for standardization)

ROLL DRIED EXTRACTS

For Use in Water Systems

SeaKem DC, (medium water gel kappa type)

SeaKem 3 (low water gel kappa type)

For Use in Milk Systems

SeaKem C, D, DC (high milk reactive kappa type)

SeaKem 9 (medium milk gel kappa type)

SeaKem 14 (high milk gel kappa type)

SeaKem L CM (very low milk gel lambda type)

ADDITIONAL PRODUCTS MANUFACTURED BY

MARINE COLLOIDS, INC.

CARRAGEENAN COMPOSITIONS

SeaKem 102	-	Kappa type carrageenan, potassium chloride
SeaKem 202	-	Kappa type carrageenan, locust bean gum, potassium chloride
Nutricol GF	-	" " "
SeaGel DG	-	Iota type carrageenan, clarified locust bean gum

TYPICAL SPECIALTY PRODUCTS

Pregelatinized locust bean gum

Clarified locust bean gum

Agarose

TYPICAL CARRAGEENAN WATER APPLICATIONS

USE	FUNCTION	PRODUCT	APPROXIMATE LEVEL
Water dessert gels (Dry powders, finished gels)	Setting agent	Gelcarin DG plus Gelcarin H WG	0.70%
		Gelcarin/Locust Bean Gum Combinations	<0.70%
Dietetic jellies	Setting agent	"	0.50%
Pie filling (chiffon, meringue)	Setting agent	SeaKem DC	0.50%
Syrups (chocolate, maple, etc.)	Bodying, suspension	SeaKem 402, Viscarin 402, Gelcarin L WG	0.20%
Fruit drink powders and frozen concentrates	Bodying & pulping effects	Viscarin Gelcarin H WG	0.50%
Imitation coffee creams	Emulsion stabilization	SeaKem 402, Viscarin 402	<0.20%
Relishes, pizza & barbecue sauces	Bodying	SeaKem 5, others	<0.50%
Buttered sauces for frozen vegetables	Cling, uniform color, mouthfeel	Viscarin	<0.1%
Soups	Bodying, gelling	Viscarin Gelcarin SI, others	0.2 - 1
Toothpaste, lotions, creams	Bodying, emulsion stabilization	Viscarin	<1.0%
Suspensions (graphite, clay, etc.)	Suspending	Gelcarin SI	<1.0%

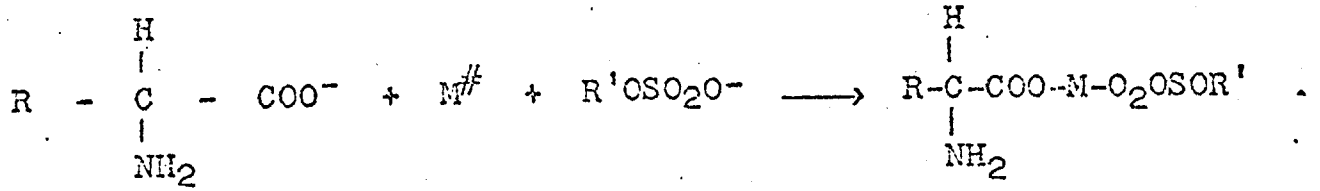
TYPICAL CARRAGEENAN WATER APPLICATIONS

B

USE	FUNCTION	CARRAGEENAN TYPE	APPROXIMATE U: LEVEL
WATER DESSERT GELS (Dry powders, finished gels)	Setting agent	Kappa & Iota	0.70%
		Kappa & clarified locust bean gum	0.70%
Dietetic jellies	Setting agent	" "	0.50%
Pie fillings (chiffon, meringue)	Setting agent	Kappa	0.50%
Syrups (chocolate, maple, etc.)	Bodying, suspension	Lambda	0.20%
Fruit drink powders and frozen concentrates	Bodying & pulping effects	Lambda & Kappa	0.50%
Imitation coffee creams	Emulsion stabilization	Lambda	0.20%
Relishes, pizza & barbecue sauces	Bodying	Kappa	0.50%
Buttered sauces for frozen vegetables	Cling, uniform color, mouthfeel	Sodium Kappa or Lambda	0.1%
Soups	Bodying, gelling	Kappa, Lambda or Iota	0.2 - 1.
Toothpaste, lotions, creams	Bodying, emulsion stabilization	Sodium Kappa	1.0%
Suspensions (graphite, clay, etc.)	Suspending	Iota	1.0%

PROTEIN REACTIVITY OF CARRAGEENAN

Above isoelectric point:



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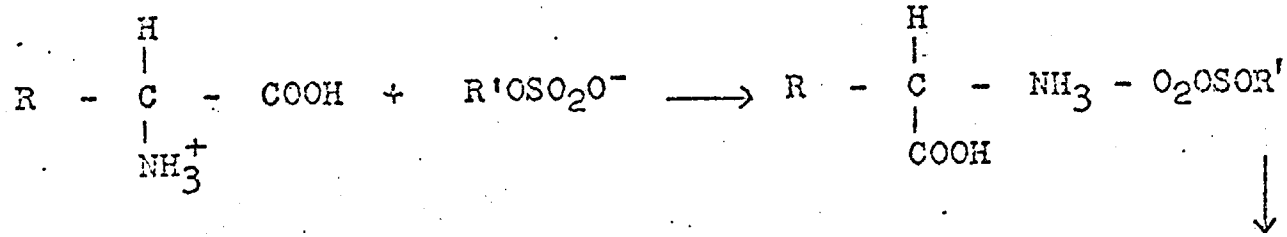
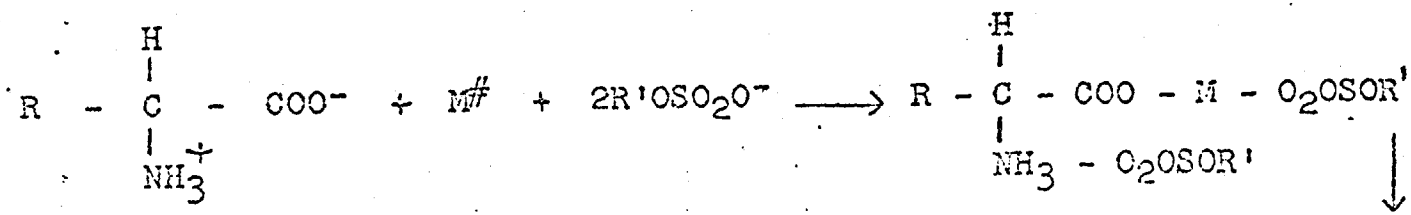


At isoelectric point:



TYPICAL CARRAGEENAN MILK APPLICATIONS

USE	FUNCTION	PRODUCT	APPROXIMATE USE LEVEL
ICE CREAM	Prevent whey separation	SeaKem DC	0.015%
EVAPORATED MILKS			
Chocolate, eggnog, fruit flavored	Suspension, bodying	Gelcarin H MR SeaKem DC	0.027% 0.027%
STERILIZED MILKS			
a) Chocolate, eggnog, etc.	Suspension, bodying	SeaKem 2 Gelcarin MAC	300 ppm
b) Evaporated (in-can (aseptic	Fat stabilization " "	Gelcarin H MR " "	25 - 50 ppm 100 ppm
c) 900-calorie diet drinks	Suspension, bodying	Gelcarin MAC	250 ppm
d) Infant formulations (Concentrates & single strength)	Stabilization of fat and protein	SeaKem 2	300 ppm

TYPICAL CARRAGEENAN MILK APPLICATIONS

USE	FUNCTION	PRODUCT	APPROXIMATE USE LEVEL
PUDDINGS & PIE FILLINGS (dry powders, finished and frozen types)			
a) Cold set without starch	Setting agent	Viscarin 402	0.5 - 1.0%
b) Cold set with starch	Setting, syneresis inhibiting	Viscarin 402 SeaKem 306, others	0.1 - 0.5%
c) Cooked flan or custard	Setting agent	SeaKem DC plus Gelcarin SI	0.3%
		SeaKem DC plus Gelcarin DG	
d) Cooked starch type	Anti-cracking, better unmolding, non-critical cooking	SeaKem DC	0.05%
WHIPPED PRODUCTS (Dry, finished, frozen, aerosol			
Creams, toppings, desserts	Fat and foam stabilization, setting	Nutricol 306 Viscarin 402, others	0.05 - 0.5%
COLD PREPARED MILK POWDERS			
Thickened drinks, shakes	Bodying, stabilizing overrun	SeaKem L CM	0.1 - 0.3%

B

TYPICAL CARRAGEENAN MILK APPLICATIONS

USE	FUNCTION	CARRAGEENAN TYPE	APPROXIMATE USE LEVEL
PUDDINGS & PIE FILLINGS (Dry powders, finished and frozen types)			
a) Cold set without starch	Setting agent	Lambda	0.5 - 1.0%
b) Cold set with starch	Setting, syneresis inhibiting	Lambda	0.1 - 0.5%
c) Cooked flan or custard	Setting agent	Kappa & Iota	0.3%
d) Cooked starch type	Anti-cracking, better unmolding, non-critical cooking	Kappa	0.05%
WHIPPED PRODUCTS (Dry, finished, frozen, aerosol)			
Creams, toppings, desserts	Fat and foam stabilization	Lambda	0.05 - 0.5%
COLD PREPARED MILK POWDERS			
Thickened drinks, shakes	Bodying, stabilizing overrun	Lambda	0.1 - 0.3%